

Effects of Different Salts on Mixing and Extension Parameters on a Diverse Group of Wheat Cultivars Using 2-g Mixograph and Extensigraph Methods

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ABSTRACT

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Cations of differing chaotropic capacities (LiCl, NaCl, and KCl) were used in small-scale mixing and extensigraph studies to assess functional changes in dough behavior of wheat cultivars varying in total protein content and HMW glutenin composition. Salt addition, regardless of cationic type, caused an increase in dough strength and stability. The smaller (hydrated) and least chaotropic cations ($\text{Li}^+ < \text{Na}^+ < \text{K}^+$) effected the greatest increase in mixing time (MT) and resistance to extension (R_{max}) and produced the most stable resistance breakdown (RBD). The effects of different cations on mixing and extensions indicated strong intercultivar variation; differential responses to salt addition were further shown when the cultivars were grouped according to protein content and *Glu-1D* or

Glu-1B genome composition. Increases in dough strength parameters due to the addition of salt were consistently more significant for cultivars showing an overexpression of Bx7 (>12% protein). In the absence of genotypic variation, a significant interactive effect of cultivar type, protein amount, and salt addition was found for all functional dough parameters except extensibility. During mixing, there was a decrease in the amount of apparent unextractable polymeric protein (%UPP) in the dough. This phenomenon was ameliorated by the presence of salt in doughs formed from weaker flours and was most pronounced early on in the mixing process ($t = 100\text{--}200$ sec). Results show the importance of refining 2-g mixograph studies to include salt in the “flour and water” dough formula.

In addition to being a flavor-enhancer in baked products, common salt also has profound effects on the physical properties of doughs. Salts, as true for any additive, alter the functional properties of the dough system.

From the beginning of the last century, cereal chemists have been aware of the ability of salt solutions of varying concentrations to solubilize wheat proteins (Gortner et al 1929). The interaction of salt ions with gluten proteins governs functional rheological parameters and, as a result, the dough-handling properties (Hlynka 1962; Tanaka and Tipples 1969; Danno and Hosney 1982). Following the addition of water to flour in mixing, the rate of starch and protein hydration determine mixing curve characteristics. In early farinograph measurements (Sandstedt 1955; Yamazaki 1955; Greer and Stewart 1959), starch and protein compete for water, depending on the degree of damaged starch in the flour. The introduction of salt into this system gave a better definition of the effects of starch-protein hydration (Tanaka and Tipples 1969). Addition of 2% salt decreased the hydration capacity of gluten by $\approx 8\%$ while having no effect on the hydration of starch (Bushuk and Hlynka 1964). It was suggested that salt occupies sites normally taken up by “bound” water; thus there may be an increase in the amount of “free” water present due to the modified gluten structure. Farinograph and extensigraph measurements have shown that salt generally increased dough development time, resistance to extension, and dough extensibility (Hlynka 1962; Tanaka and Tipples 1969; Galal et al 1978; Preston 1989).

Different studies have shown that gluten sensitivity to salt is cultivar-specific (Gortner et al 1929; Huebner 1970; Arakawa and Yonezawa 1975) and that polymeric glutenins with a higher proportion of α -type HMW glutenin subunits are more sensitive to salt (Kim and Bushuk 1995). This increased sensitivity is thought to be due to amino acid composition and subsequent conformational differences in tertiary protein structure (Galal et al 1978; Kim and Bushuk 1995).

Preston (1989) showed that uncharged salts influence hydrophobic and electrostatic interactions causing conformational changes in gluten proteins. Salts induce an electrostatic shielding of ionic amino acids on the surface of gluten proteins, thus reducing electrostatic repulsion or attraction of the proteins and inducing hydrophobic

aggregation. Ultimately, these cohesive forces determine the physical dough properties of wheat flours dependent on the concentration and type of salt anion added. Low salt levels (0.05–0.1M) increased dough strength, and these effects were more pronounced in the presence of the more chaotropic anions SCN^- and I^- . Chaotropic anions at higher ionic concentrations (0.5–1.0M) caused reduced dough strength, unlike nonchaotropic anions (Cl^- , Br^-), which increased dough strength. At high ionic strengths (>0.5M) the interprotein electrostatic interactions are swamped by the large amount of ions present and hydrophobic interactions are thought to be responsible for changes in protein properties. At lower ionic concentrations (<0.5M), ionic interactions predominate and determine the solubility and aggregation of gluten proteins (Preston 1981; He et al 1992). Cations have been dismissed in the past as having less impact on dough functionality than anions, or their effects on functional properties have only been briefly mentioned (He et al 1992). Our preliminary research showed that the effect of different cations on dough properties produced many systematic changes, which may provide insight into the nature of bonding in dough systems. Furthermore, this study emphasizes the need to include NaCl in the standard method for small-scale mixing, especially in light of the fact that the current standard method for extension testing and baking requires NaCl.

MATERIALS AND METHODS

Cultivars

Cultivars from North America (Glenlea* and Red River*) and Australia (Janz, Frame, Ouyen, Meering, Chara*, Kukri*, Halberd, Goldmark, Rosella, Spear) were used in this study; cultivars marked with an asterisk show a naturally occurring overexpression of subunit *Glu-1Bx7* (OE Bx7). The cultivars chosen varied in *Glu-1A* and *Glu-1D* composition, protein composition, and *Glu-1B* x/y ratio; all cultivars were *Glu-1Bb* (7+8) except for Halberd (*Glu-1Be*) and Spear (*Glu-1Bc*) (Table I). Two dark northern spring (DNS) samples were also included in the evaluation of the effect of different salts (alkali metal cations) on functional dough properties.

Grain and Flour Protein Evaluation

Initial grain protein and moisture content was measured using a near-infrared (NIR) analyzer (Grainspec, Foss UK Ltd, York) before overnight conditioning. The grain was milled to flour in a Brabender Quadramat Jr. mill, and moisture and protein determinations were made with a NIR spectrometer (6500, Foss Systems, Silver Spring, MD). The *Glu-1B* x/y ratio and HMW/LMW glutenin ratio were quantified by reversed-phase HPLC (RF-HPLC) (Marchylo et al

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1989). The proportions of gliadin and glutenin, together with the percentage of unextractable polymeric protein (%UPP) in endosperm protein was determined using a modified method (Larroque et al 1997) for wheat protein analysis by size-exclusion HPLC (SE-HPLC) (Batey et al 1991). To gain an insight into the processes occurring on mixing and dough breakdown, samples of dough were taken during mixing at different time intervals, immediately frozen, and subsequently freeze-dried. The samples were later ground to a uniform fine powder and the SE-HPLC procedure was performed. The resultant measurement of the size of the polymer was termed apparent %UPP.

Functional Dough Properties

A 2-g mixograph (Rath et al 1990) was used to evaluate functional dough properties. The water absorption was estimated by Approved Method 54-40A (AACC 2000) using the protein and moisture contents of the flour. Mixing was performed in duplicate and parameters measured were mean time to peak dough development (mixing time, MT [sec]); height of mixograph trace at peak resistance (PR [au]); bandwidth at peak resistance (BWPR [au]), and percentage decrease in dough resistance 3 min after the peak (resistance breakdown, RBD [%]).

Extensigraph measurements were made (two to four repeats per sample) on 1.7-g dough pieces to determine the maximum resistance of the dough to extension (R_{max} [N]) (Gras and Bekes 1996) and dough extension before severing (Ext [m]), calculated with custom software (Rath et al 1994).

Addition of Salt

Mixing was done using a 2-g mixograph in the absence of salt and in the presence of a 2% NaCl (flour basis) solution. Mixes were then repeated with equimolar solutions (1.15M stock solutions) of KCl and LiCl; a constant amount of each salt solution was added for each mix performed. Extensigraph measurements were made with water, NaCl, KCl, and LiCl using the respective optimal mixing times for each solution. To ensure that the added salt solutions significantly increased the original cation concentrations of the flour, the concentrations of the major elements in the flour samples, sodium and potassium, were analyzed by ICP (AGAL, NSW Method NT2.46).

Analysis of Glutenin Polymer Size in Developing Dough

To estimate how the glutenin polymer size in dough changes during mixing under different salt conditions, dough samples (<500 mg) were taken at MT-20%, MT, MT+20%, and at the end of the 10-min mixing process. Samples were freeze-dried and ground with a pestle and mortar. The dried dough was then analyzed for apparent %UPP using SE-HPLC as described above (Larroque et al 1997).

Statistical Analysis

All samples were measured in duplicate (mixograph) and triplicate (extensigraph). Analysis of variance and Student's *t* test were performed using the MSUSTAT computer package (v. 4.1, Montana State University, Bozeman, MT).

Initially, the overall effect of the presence of salt and salt in various cationic forms, was established using a one-way ANOVA and comparison of means. The samples examined were then grouped according to cultivar, protein content (%P), D genome composition, and OE Bx7, as defined by *Glu-1B* x/y ratio > 3.5, and a comparison of means was performed. A two-way ANOVA was established to see whether the significant differences found from the Students' *t* test were due to an interaction of salt with a particular variable such as cultivar, D genome composition, or presence-absence of OE Bx7. A fourth group variable was established to compare the effects of salt on cultivars of different protein contents; cultivars with >12% protein represented the classification of Australian Prime Hard wheat and were compared to cultivars with <12% representing Australian Hard, Premium White, and Standard White wheat. Bearing in mind that the cultivars examined were not identical within these groupings, these *t*-test results and two-way ANOVA are of value when generalizing which cultivars are more susceptible to the effects of salt. A more accurate picture of these effects could be obtained from four of the cultivars (Janz, Frame, Meering, and Ouyen) grown in two different environments, producing two protein contents apiece (9 and 12%); flour samples were kindly provided by AWB Ltd. (Victoria, Australia). Using this data, a three-way ANOVA was established to test whether the interactive effect of different salts on cultivars was related to protein content.

RESULTS AND DISCUSSION

Cultivar Characteristics

Endogenous levels of sodium and potassium cations are a function of plant nutrition and assimilation capabilities; level ranges were 0.006–0.041 mg of Na⁺/g and 2.31–3.67 mg of K⁺/g. The amount of Na⁺ and K⁺ cations added during the experiments were respectively 10² to 10³× and 10× as much as endogenous cation levels. We were therefore confident that the effects generated were due to added Na⁺ and K⁺ and not to differences found within the flour itself. The protein content and composition of the different flours varied widely (Table I). Weaker flours used more in cake and biscuit making, such as Rosella, Halberd, and Spear, showed significantly lower %UPP (29.81–34.63) than the stronger breadmaking flours Janz, Ouyen, Frame, and Meering (46.27–50.72). Those flours showing an overexpression of *Glu-1* Bx7, and consequently the highest *Glu-1B* x/y ratios (>3.5), had the largest %UPP (54.3–57.7) values.

TABLE I
Protein Content and Composition of Cultivars^a

Cultivar	HMW-GS			Protein (%)	UPP ^b (%)	Glutenin/Gliadin	<i>Glu-1</i> Bx/By	HMW/LMW
	<i>Glu-1A</i>	<i>Glu-1B</i>	<i>Glu-1D</i>					
Janz	1	7+8	2+12	9.6 (11.9)	47.44 (50.72)	0.92 (0.78)	3.33 (2.92)	0.39 (0.44)
Ouyen	2*	7+8	2+12	9.0 (12.2)	46.27 (49.93)	0.94 (0.92)	2.95 (2.87)	0.38 (0.43)
Meering	2*	7+8	2+12	8.8 (12.6)	45.48 (48.45)	0.87 (0.83)	3.01 (2.57)	0.38 (0.46)
Rosella	2*	7+8	2+12	8.7	29.81	na	3.10	na
Goldmark	2*	7+8	2+12	10.3	44.23	na	2.10	na
Chara	2*	7+8	2+12	12.1	54.61	0.88	4.59	0.50
Frame	1	7+8	5+10	9.2 (12.7)	48.22 (47.60)	0.83 (0.79)	3.11 (3.17)	0.37 (0.39)
Kukri	1	7+8	5+10	12.2	56.13	0.90	7.45	0.42
Red River	1	7+8	5+10	14.2	54.34	0.85	5.59	0.42
Glenlea	2*	7+8	5+10	13.3	57.72	0.82	6.12	0.52
Halberd	1	20	5+10	8.4	30.35	na	na	na
Spear	1	7+9	5+10	11	34.63	na	2.56	na

^a *Glu-1* Bx subunits in bold type indicate an overexpression of *Glu-1* Bx7. Where two values are given for a cultivar, the first value indicates low % protein and the value in parentheses indicates high % protein. Average values shown (standard error < 5% average value); na, not applicable.

^b Apparent unextractable polymeric protein.

There was more of an overlap in HMW/LMW and glutenin/ gliadin ratios between the weak, strong, and overstrong cultivars. Extra-strong mixing characteristics have been reported for cultivars with OE Bx7, such as Glenlea (Hussain et al 1998) and Red River (D'Ovidio et al 1997). We demonstrate here that the recently released Australian cultivars, Kukri and Chara, which also show an over-expression of *Glu-1Bx7* (and hence a Bx/By ratio > 3.5) also are significantly stronger than non-OE Bx7 cultivars, regardless of salt addition.

Effects of Different Salts on Mixing and Extension Parameters

Salt addition significantly affected all functional dough parameters ($P < 0.001$) and, based on Table II, the salt type also had significant effects on almost all mixing and extensigraph parameters. The addition of different salts caused changes in the mixing and extension properties of flours from different cultivars (Figs. 1–4). Salt addition, regardless of cationic type, caused an increase in mixing time (Fig. 1). This is consistent with previous findings obtained using the farinograph (Hlynka 1962; Galal et al 1978) and 10-g mixograph (Danno and Hosney 1982). The effects of different cations on mixing indicated strong intercultural variation with a range of responses to different cations. For example, some cultivars showed large increases in MT with cations of increasing size (Ouyen, 9% protein), whereas others did not show any differentiation in MT due to the different cations (Rosella, Frame, 9%). Cultivars chosen at two different protein levels provided an opportunity to see how protein levels affect mixing in the absence of genotypic variation. Generally, the MT response pattern to the addition of different cations was the same for low and high protein contents, with the exception of the relatively higher MT values found with NaCl addition to Janz, Meering, Frame, and Ouyen of lower protein content (%P) (Fig. 1A). Higher MT values were also achieved in low %P Janz

and Frame with the addition of Li^+ and K^+ respectively. The effect of salt addition was more dramatic for RBD. For example, for Frame (9%), RBD dropped from 24 to 9% with the addition of NaCl (Fig. 2A); dough strength was significantly increased. The PR and BWPR profiles (Tables II and III) of different cultivars also showed a variable response to the addition of different salts. In some cases, LiCl even caused a decrease in PR (Ouyen 9%, Janz 9%, Frame 12%) and BWPR (Rosella, Goldmark, Spear). Figure 3B indicates how those cultivars with higher protein contents form stronger doughs (higher R_{max}) than low protein cultivars (Fig. 3A) and that this was enhanced in most cases by the addition of NaCl and KCl. It was more difficult to discern differences in the effects of cations on Ext (Fig. 4), this may well be due to reproducibility problems inherent in the methodology (Allen et al 2000). However, some strong cultivars (Glenlea and Red River) did not respond positively to the addition of salt and their Ext values actually decreased in the presence of salt (Fig. 4B).

A comparison of means (Table II) shows that, in general, the larger and least chaotrophic the cation added, the greater the increase in MT ($\text{Li}^+ < \text{Na}^+ < \text{K}^+$). This order of ionic interaction within the dough matrix is thought to be a function of the steric integration of the hydrated ions. Assuming that there is little free or bulk-phase water in the gluten phase of the dough, it is possible that it is sterically easier for the smaller $\text{K}^+(\text{aq})$ ion to enter that phase and then to accept protein ligands. Significant increases in PR, BWPR, and R_{max} with salt also occurred in the same order ($\text{Li}^+ < \text{Na}^+ < \text{K}^+$), though the effect was not significantly different for NaCl and KCl. The decrease in RBD with salt was the same, regardless of the cation type. It is noteworthy that only NaCl significantly increased dough extension, whereas LiCl significantly decreased extensibility. This would be an important factor when dough formulations are being devised, as extensibility is an important criterion in dough quality.

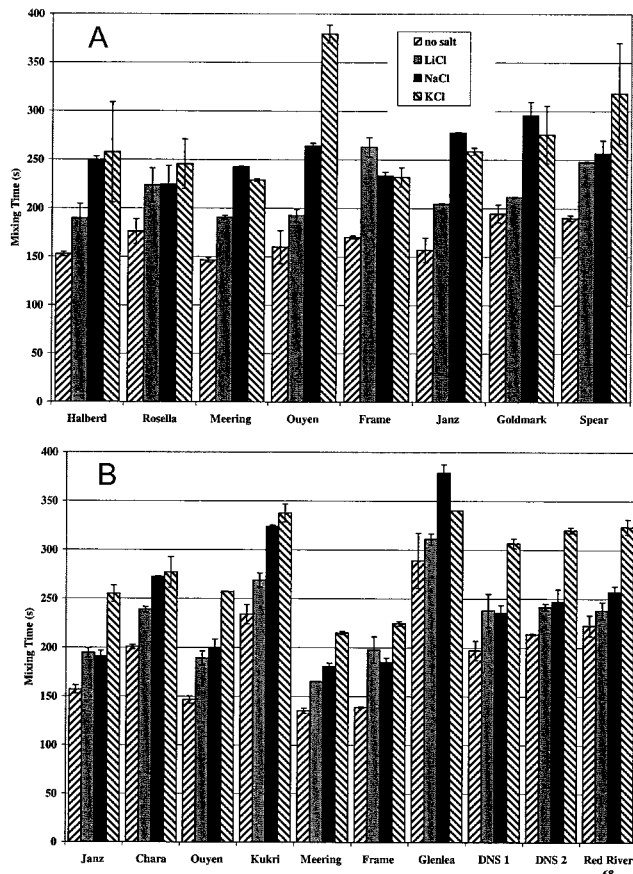


Fig. 1. Effect of different salts on mixing time of cultivars with (A) low protein content (8.7–11% P) and (B) high protein content (11.9–14.2% P).

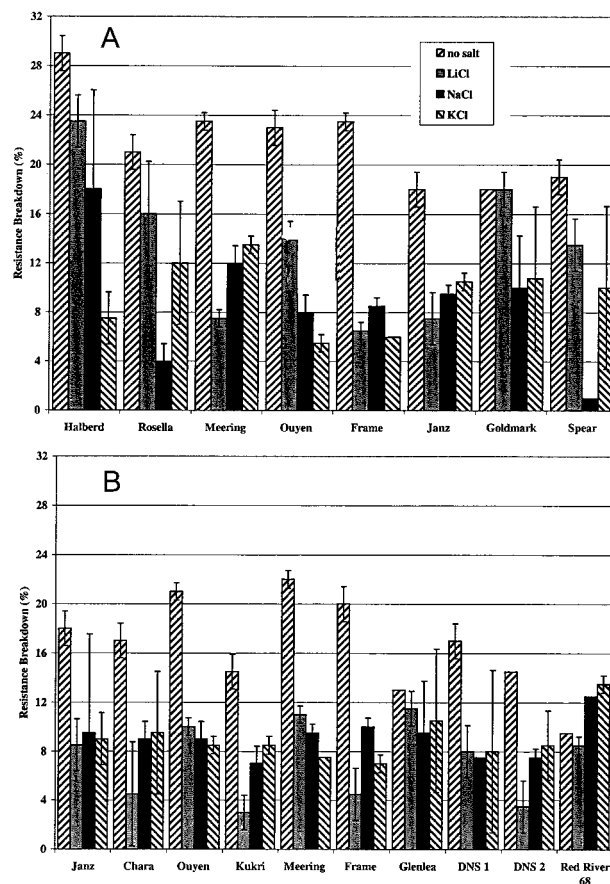


Fig. 2. Effect of different salts on resistance breakdown of cultivars with (A) low protein content (8.7–11% P) and (B) high protein content (11.9–14.2% P).

As noted earlier, water absorption was determined using protein and flour moisture content and to standardize the method for comparison of mixographs, a constant anion concentration was added for each mix. We are mindful that the use of different salts is a complex issue for rheologists because ions differ in binding capacities and may alter the amount of water available to the flour constituents. Taking into account the sphere of hydration of each ion, whereby the hydrated radius of Li^+ (0.382 nm) $>$ Na^+ (0.358 nm) $>$ K^+ (0.331 nm) (Nightingale 1959), water molecules, involved in salt bridging for example, would actually be more tightly bound by Li^+ . It is therefore expected that the availability of water for interactions with other flour components would be lowest for Li^+ and, if anything, it could be anticipated that the addition of LiCl would create stronger doughs. This was not the case, as summarized in Table II. Alterations in the structure of the dough complex on addition of different cations (at constant metal ion concentrations) are likely to be due to coordination of the cations with protein functional groups. Differences in further statistical analyses were determined without differentiating the effects of different salt types.

Cultivars were grouped (Table III) to evaluate the effects of protein, D genome, and OE Bx7, on functional dough properties to see whether salt affects one type of cultivar more than another. The functional dough parameters of cultivars with OE Bx7 or a protein composition $>12\%$ ($>12\%$ P), showed significantly higher PR, BWPR, and Ext, with lower RBD values than cultivars without OE Bx7 or with $<12\%$ P, respectively. However a D genome composition of 5+10 (*Glu-1Dd*) and OE Bx7 showed a significantly increased MT compared with a 2+12 (*Glu-1Da*) D genome. R_{max} was significantly higher for $>12\%$ P, *Glu-1Dd* and OE Bx7 than for $<12\%$ P, *Glu-1Da*, and normal expression of *Glu-1B*, respectively. When the combination of the effect of salt (NaCl , KCl , or LiCl) together with the differences in cultivar type was assessed, a highly significant interaction ($P < 0.001$) for all parameters was also found. When the cultivars were grouped according to whether or not they had $>12\%$ P, there was an interactive effect with salt for these parameters ($P < 0.001$) in the order of significance: $\text{BWPR} > \text{RBD} > \text{PR} \equiv \text{Ext} \equiv R_{\text{max}}$. No significant interaction was found for MT. There was no statistically significant

TABLE II
Differences in Mixing and Extension Properties^a for Different Cationic Salt Treatments

Treatment	n	MT	PR	BWPR	RBD	n	EXT	R_{max}
No salt	42	194.0a ^b	340.0a	211.1a	17.90b	61	0.132b	0.2502a
LiCl	42	222.0b	411.0b	298.6b	10.07a	79	0.1257a	0.2871b
NaCl	44	251.8c	436.5b	347.8c	9.81a	78	0.1445c	0.3448c
KCl	48	286.1d	417.4b	348.9c	9.56a	81	0.1285ab	0.3467c
Mean		240.3	402.2	303.7	11.7		0.1	0.3
LSD		19.3	34.1	29.0	1.9		4.1e	1.0e

^a MT, mixing time; PR, peak resistance; BWPR, bandwidth at peak resistance; RBD, resistance breakdown; EXT, dough extension; R_{max} , resistance to extension.

^b Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE III
Differences in Mixing and Extension Properties^a for Different Cationic Salt Treatments and Cultivars

Variable	n	MT	PR	BWPR	RBD	n	EXT	R_{max}
Cultivar								
Frame (9%)	8	224.6b–f ^b	376.3de	286.6c–e	11.13b–d	13	0.116b	0.315f
Frame (12%)	8	186.4ab	448.9f–h	297.5d–f	10.38a–c	13	0.124bc	0.382hi
Janz (9%)	8	199.4a–c	465.6f–h	327.5d–h	11.38c–e	14	0.127c–e	0.365gh
Janz (12%)	8	224.4b–f	333.4b–d	270.6cd	11.25b–d	14	0.126cd	0.293d–f
Meering (9%)	8	173.9a	460.8f–h	313.9d–g	14.13e	14	0.142gh	0.287de
Meering (12%)	8	202.1a–d	324.5bc	263.4b–d	12.5c–e	13	0.124bc	0.236c
Ouyen (9%)	8	198.1ab	474.3gh	329.4d–g	12.63c–e	14	0.136e–g	0.351g
Ouyen (12%)	8	249e–g	361.8cd	265.3b–d	12.13c–e	14	0.121bc	0.276d
DNS 1	8	244.3c–f	419.5ef	284.8c–e	10.13a–c	14	0.126cd	0.310ef
DNS 2	8	255.5e–g	429.8fg	288.4c–e	8.5ab	14	0.135d–g	0.352g
Chara	8	247.3d–g	480.9h	371.5gh	10a–c	13	0.138fg	0.389i
Kukri	8	291.1gh	435.3f–h	344.8e–h	8.25a	14	0.124bc	0.413j
Glenlea	8	329.8h	481.8h	389.5h	11.13b–d	15	0.15h	0.397ij
Red River1	8	260.3fg	461.5f–h	359.1f–h	11a–d	14	0.13c–f	0.451k
Rosella	8	212.9a–e	271a	184.9a	13.5de	14	0.15h	0.128a
Halberd	10	212a–e	290.9ab	273.5cd	20.88f	16	0.124bc	0.173b
Goldmark	8	245.8d–g	341.5cd	198.4ab	14.19e	16	0.099a	0.225c
Spear	8	256e–g	368cd	225.8a–c	10.88a–d	16	0.123bc	0.225c
LSD		15.14	9.17	48.17	2.85		0.009	0.024
Protein content								
<12% P	72	229.8a	337.2a	275.4a	13.62b	115	0.123a	0.2318a
>12% P	80	238.6a	455.8b	330.6b	10.52a	116	0.133b	0.3783b
LSD		14.22	15.16	18.27	1.39		0.010	0.02
D genome composition								
2+12	54	216.2a	409.3a	309a	12.37a	77	0.131a	0.2791a
5+10	50	256.1b	413.9a	327.3a	12.04a	83	0.130a	0.3783b
LSD		17.41	21.99	28.39	2.07		0.010	na
<i>Glu-1B</i> ($>12\%$ P)								
Non-OE Bx7	54	214.4a	422a	306.5a	12.03b	73	0.123a	0.283a
OE Bx7	32	282.1b	464.8b	366.5b	10.09a	55	0.135b	0.4063b
LSD		17.45	25.76	25.56	1.56		0.010	0.03
Mean		240.26	402.17	303.73	11.744		0.133	0.310

^a MT, mixing time; PR, peak resistance; BWPR, bandwidth at peak resistance; RBD, resistance breakdown; EXT, dough extension; R_{max} , resistance to extension; OE, overexpressing; na, not applicable.

^b Values followed by the same letter are not significantly different ($P < 0.05$).

interaction between the effects of salt on cultivars differing in D genome (*Glu-1Dd* or *Glu-1Da*), though there was a highly significant ($P < 0.001$) interaction of salt with lines with OE Bx7 for certain functional parameters (PR, RBD, and R_{max}). In summary, salt affected all cultivars differently, and the most significant effect in dough strength was achieved with cultivars showing OE Bx7, regardless of D genome composition. Kim and Bushuk (1995) found that cultivar differences due to the relative proportion of x-type/y-type high molecular weight glutenin subunits are critical in salt-sensitivity. Cultivars with a high ratio of x-type/y-type subunits (such as Glenlea) had HMW-GS that were easily aggregated or precipitated by salt and therefore were thought more salt-sensitive. A similar overall response to salt addition was found in our research, which encompassed more widely varying cultivars.

Interactive Effects of Protein Content, Salt, and Cultivar Type on Dough Properties

Combined effects of protein, salt, and cultivar could be investigated in a three-way ANOVA using cultivars (Janz, Frame, Meering, and Ouyen) differing only in protein content. A significant interaction was found for all parameters except extensibility. (Table IV). Interestingly, the addition of salt (NaCl, KCl, or LiCl) alone significantly ($P < 0.001$) altered dough strength (MT, RBD, and R_{max}), BWPR and Ext to a greater extent than any other parameter alone, with the exception of the highly significant effect of protein on PR.

Differences were also observed in the protein composition of Janz, Frame, Meering, and Ouyen at the two protein levels (Table I). Though higher protein levels were accompanied by an increase in HMW/LMW glutenin ratio, there was a decrease in glutenin/gliadin

ratio, and Frame even showed a decrease in %UPP at the higher protein level (Table I). One explanation given for this comes anecdotally from local growers that the average temperature during that growing season was considerably cooler. This could have implications on gluten protein composition as it has been noted in several investigations of G×E effects that gliadins are more susceptible than glutenins to environmental stress (Graybosch et al 1995; Panozzo and Eagles 2000) and that susceptibility to stress is cultivar-specific.

Although in this study we concentrated on the use of equimolar monovalent cations to alter dough properties, in preliminary studies (not shown) we found that when the divalent cation $CaCl_2$ was substituted for NaCl, the resulting mixing and extensigraph parameters were largely unchanged or only slightly higher than control values. This was also shown by He et al (1992). Both the divalent Ca^{2+} ion and Li^+ ion have larger hydrated radii with a greater charge density than both Na^+ and K^+ . This would enable stronger ion-water bonding and conversely weaker binding with gluten proteins. Thus Ca^{2+} , being more chaotropic, decreased the strength of the ionic bonds, possibly due to the formation of internal salt bridges or coordination.

The distinct divergences in dough rheology due to different cationic salts caused inherent changes in parameter correlations on the addition of different cations. Accepted cereal chemistry relationships (Sapirstein and Fu 1998; Uthayakumaran et al 2000) were altered, for example between %UPP and R_{max} , when different cultivars were mixed in the absence of salt or presence of LiCl, NaCl, or KCl ($r^2 = 0.49, 0.47, 0.33,$ and $0.36,$ respectively). Likewise, the correlation between Bx/By and MT was significant in the absence of salt ($r^2 = 0.54$), yet was reduced in the presence of salt.

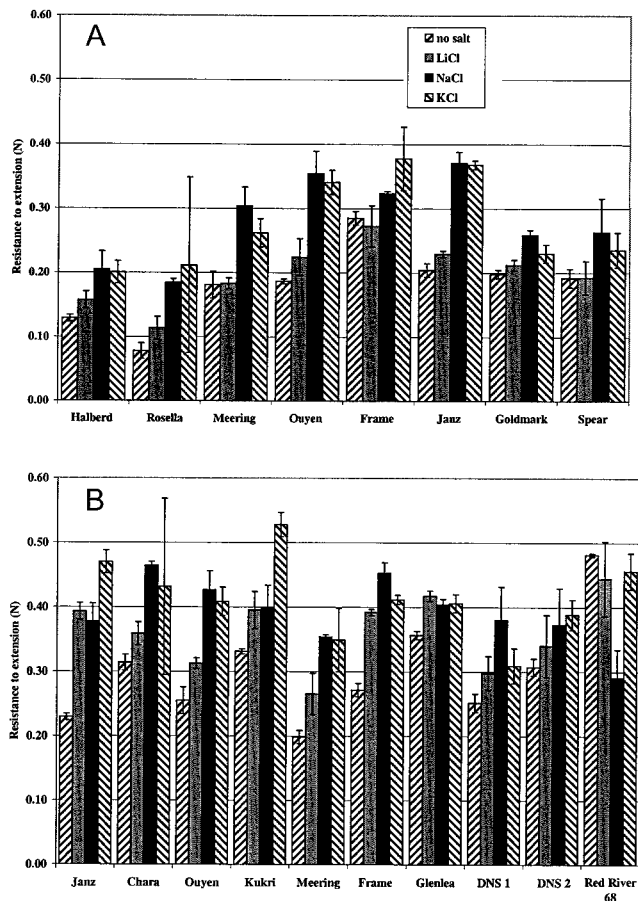


Fig. 3. Effect of different salts on maximum resistance to extension of cultivars with (A) low protein content (8.7–11% P) and (B) high protein content (11.9–14.2% P).

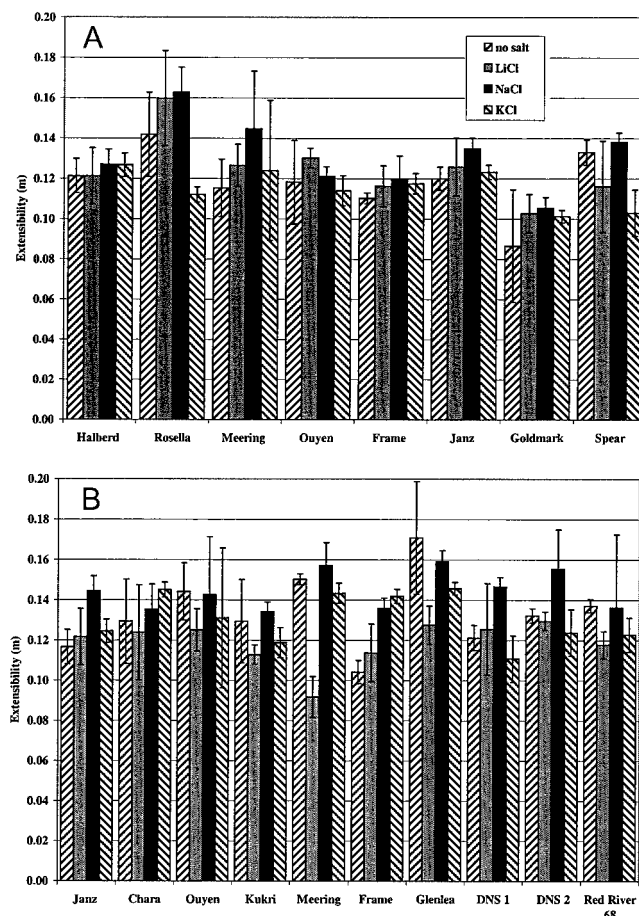


Fig. 4. Effect of different salts on extensibility of cultivars with (A) low protein content (8.7–11% P) and (B) high protein content (11.9–14.2% P).

Changes in Apparent %UPP on Mixing with Different Salts

To ascertain how different cations affect functional behavior, the degree of protein polymerization was assessed during mixing. The initial amount of unextractable polymeric protein (of flour before hydration and mixing) of those weaker cultivars (with low %UPP) such as Rosella and Halberd (Fig. 5A,B), gave shorter mixing times as less polymeric protein was present in the gluten matrix to sustain resistance to mixing. Stronger flours such as Janz and Frame had a higher %UPP and correspondingly longer MT (Fig. 5C,D) and the extra-strong flours, Kukri and Glenlea (Fig. 5E,F), had the longest MT. In agreement with previous findings, the amount of polymeric protein decreased immediately on hydration and mixing (Weegels et al 1994; Skerritt et al 1999) and the decrease in %UPP was faster in weak dough cultivars than in those yielding strong dough (Skerritt et al 1994; Bushuk et al 1997). The presence of salts in doughs formed from weaker flours caused a decrease in the rate of breakdown of the polymeric protein during mixing. This would explain how the addition of salts appears to strengthen doughs: by complexing the polymeric protein and allowing more resistance to mixing. This was most pronounced early on ($t = 100\text{--}200$ sec) in the mixing process. The smaller cation (K^+)

slowed down the decrease in apparent %UPP due to mixing, probably due to increased interactions with protein ligands and formation of more stable structures. By comparison, stronger flours (Janz and Frame) showed a decreased rate of fall-off in %UPP during mixing and the rate of decrease was slower still for the strongest cultivars (Glenlea and Kukri). In contrast to the effect of salt on decreases in %UPP during mixing with weaker cultivars, the presence of salt during mixing of strong and extra-strong cultivars generally increased the rate of decrease in %UPP or had little effect.

When the effect of salt on microbaking was assessed (results not shown), loaves containing *Glu-1Dd* were larger than those containing *Glu-1Da*. The longer MT required with NaCl addition caused the significant increase in the volumes of loaves containing *Glu-1Dd* as the dough was more developed. Samples containing *Glu-1Da* subunits were virtually identical, regardless of whether MT_{+salt} or MT_{-salt} was used. However, studies (Holmes and Hosenev 1987; He et al 1992) have shown that salt addition may not necessarily improve loaf volume, despite causing significant increases in dough strength. In this case, salt addition to an adequately strong flour dough may simply be creating a dough with decreased plasticity that is too strong for breadmaking.

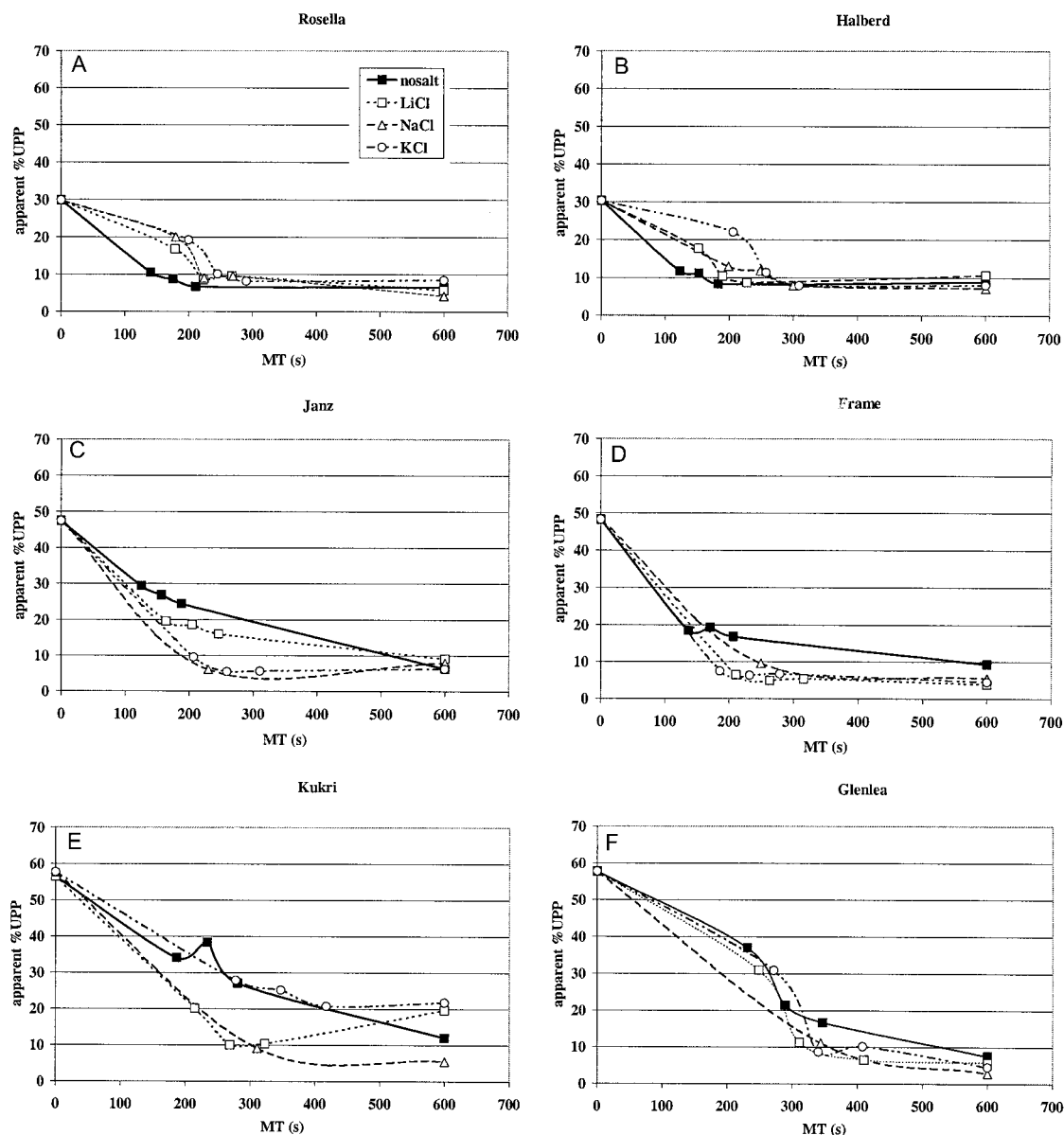


Fig. 5. Changes in apparent unextractable polymeric protein (UPP) during mixing and effect of different cationic salts on weak cultivars Rosella (A) and Halberd (B); strong cultivars: Janz (C) and Frame (D) and extra strong cultivars Kukri (E) and Glenlea (F).

TABLE IV
Three-Way ANOVA (*f*-values) for Salt Treatment with Cultivar (CV) and Protein (PRO) Content

	df	MT	PR	BWPR	RBD	df	EXT	<i>R</i> _{max}
PRO	1	489.39***	2988.28***	154.53***	5.14*	1	19.04***	90.21***
CV	3	85.13***	31.58***	1.71ns	11.83***	3	5.09**	28.74***
PRO×CV	3	13.09***	49.2***	10.53***	0.93ns	3	2.38ns	0.59ns
SALT	3	747.52***	38.06***	231.3***	344.88***	3	7.14***	108.39***
PRO×SALT	3	46.09***	6.22**	2.83*	1.31ns	3	4.86**	5.86***
CV×SALT	3	57.2***	19.81***	11.13***	8.73***	9	2.01*	2.52**
PRO×CV×SALT	3	31.6***	22.41***	4.2***	5.63***	9	1.39ns	2.55**
Residual	32					77		

a *, **, *** = significant at *P* < 0.05, 0.01, and 0.001, respectively; ns = not significant.

CONCLUSIONS

Changes shown due to the addition of salt and alteration in the relationships between functional parameters demonstrate that salt, and different cations of salt, plays an important role in determining the mixing and extension properties of dough. It is, therefore, recommended that cereal chemists and those involved in developing or testing new wheat cultivars take into account the type and amount of salt used in different formulations for end-product use. The small-scale mixograph method should be amended to contain 2% NaCl at the outset of investigations, especially when used in formulations for further extension and baking studies.

Intercultivar variations confirmed the common observation (Gupta and MacRitchie 1994) that the size distribution of polymeric protein (%UPP), *R*_{max}, and MT do not correlate significantly with flour protein among different genotypes and that the differences in functional attributes observed were due to allelic variation at *Glu-1* and *Glu-3* loci. However, for identical cultivars grown at different sites and with consequently different levels of protein, there was a significant interaction of salt, cultivar, and protein for all mixing parameters and for *R*_{max}; the interactive effect of different salts on these cultivars was indeed related to protein content. Experiments such as these present wonderful opportunities to “pull apart” the different functional parameters comprising dough strength and enable a greater understanding of the effects of protein composition on individual parameters.

The newly released Australian cultivars, Kukri and Chara, which both have an over-expression of *Glu-1Bx7*, gave overstrong doughs when mixed or extended, and were similar in properties to Glenlea and Red River. The strong rheological properties were correlated with high %UPP values and high Bx/By ratios. Salt interacted most significantly with cultivars showing overexpression of *Glu-1Bx7*, regardless of D genome composition. However, the findings also suggest that less salt would be required to achieve optimal dough extensibility with extra-strong cultivars and that this could have positive ramifications for reduced-salt bread products. Furthermore, KCl could be considered as an alternative additive to achieve enhanced dough strength in weaker flours.

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