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Analysis and Correlation Studies on Gluten Quantity and Quality During Production

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Abstract: Three factors, mixing times (5-11 min), salt (2-8%) and water levels (61.4-65.4% for strong flour and 57.5-61.5% for weak flour) were investigated during dough mixing for the production of gluten in terms of its quantity and quality and its correlation. Quantity of gluten was measured by weighing the wet gluten content obtained from doughs washed under running tap water. The wet gluten was dried using air oven drying method to obtain the dry gluten content. In terms of quality, volume expansion analysis was performed by frying the wet gluten and measuring its volume expansion using mustard seed displacement method. The extensibility of gluten was determined using a tensile test attached to an Instron 5566 machine. Results show that gluten quantity and quality measurements gave good correlations with positive coefficient of correlation (R) which are stronger for strong flour ($0.60 < R < 0.80$) than for the weak flour ($0.30 < R < 0.50$). These correlations can be used in the gluten based industry to improve the production of gluten with respect to both the quantity and quality.

Key words: Dough, analysis, volume expansion, extensibility

INTRODUCTION

The uses of wheat-gluten in industry are intensely applied as commercial products in both food and non-food industries due to the unique cohesive properties of gluten (Day *et al.*, 2006). In the food industry, wheat gluten is used in the production of breakfast cereals, crab analogues and meat substitutes while for the non-food industry, it is used as pet food, natural adhesives and biodegradable films. Wheat gluten was first developed as meat substitute many years ago in China as it has a similar look and texture of meat when cooked, thus making it a popular meat substitute (Hackett, 2007). Gluten made from wheat flour by mixing dough and washing it under the running water is fried into gluten balls and served as a traditional food in Taiwan (Chen *et al.*, 1998).

The development of gluten network during mixing is affected by several factors namely flour composition, ingredients used and the mixing process parameters (Roach *et al.*, 1992; Janssen *et al.*, 1996; Létang *et al.*, 1999; Ćurić *et al.*, 2001; Angioloni and Dalla Rosa, 2005). Gluten is obtained naturally during dough mixing from the interactions between polymers cross-links forming disulphide bonds which contribute to an increased dough

strength, maximum resistance to extension and restoring force after deformation. However, when a dough is mixed longer pass its optimum development, the cross-links of disulphide bonds will break. Whilst water plays a vital role in hydrating the protein fibrils and starting the interactions between the proteins cross-links with the disulphide bonds, there exists an optimum water level in developing a cohesive and viscoelastic dough with optimum gluten strength (Faubion and Hosney, 1989; Abang Zaidel *et al.*, 2008). Salt, the other important ingredient is reported to give strengthening or tightening effect on the gluten during dough mixing. The presence of salt can accelerate gluten formation, tighten the dough and increase the mixing time (Niman, 1981). Roach *et al.* (1992) reported that the influences of salt on protein solubility affects dough properties such as mixing requirements, optimum absorption, ease of processing and on bread quality i.e., loaf volume. The strength of gluten is largely determined by the amount of protein content in the wheat flour itself. Flour from strong wheat with higher protein content produces a higher quantity and stronger gluten compared to the weak flour. Direct studies on the effects of protein content on gluten quality was conducted by Chiang *et al.* (2006) through the

evaluation of volume expansion of fried gluten and on the extensibility of gluten was performed by Tronsmo *et al.* (2003) and Sliwinski *et al.* (2004a, c). Other studies on the effect on protein contents were applications in the breadmaking quality (Janssen *et al.*, 1996; Sliwinski *et al.*, 2004b, c). In recent years, studies on gluten properties were most commonly linked and related to evaluations in terms of breadmaking quality (Janssen *et al.*, 1996; Kokelaar *et al.*, 1996; Kieffer *et al.*, 1998; Tronsmo *et al.*, 2003; Sliwinski *et al.*, 2004b). For example, Tronsmo *et al.* (2003) suggested that the large deformation test gives good correlations between gluten and breadmaking qualities. They recommended large deformation testing as a more suitable test for gluten quality as it can be related to its eating quality. The uniaxial extension test is one of the most commonly used methods for testing the extensibility of gluten by exerting deformation on the protein cross-links in the gluten developed during mixing.

The properties of gluten produced can be measured by its quantity and quality. In similar case to wheat proteins, both the quantity and quality are important in ensuring good applications in products (Chiang *et al.*, 2005; He and Hosene, 1992; Wang *et al.*, 2007) and fetch higher price in the market (Stiegert and Blanc, 1997). A high quantity does not guarantee a good quality and as such in most cases, the total content is reported instead. The correlation approach may be used to evaluate the relationship between the quantity and quality measurements. In gluten related studies, Ćurić *et al.* (2001) determined the correlation between two different methods for gluten washing while Mann *et al.* (2008) determined the correlations between small-scale and large-scale mixing and extensional characteristics of wheat flour dough. The relationships between the two types of analyses, quantity and quality measurements of gluten were also determined using correlation studies by means of simple linear regression. Xu *et al.* (2008) investigated the relationship between creep parameters and chemical compositions of indica rice gel while Wesenbeeck *et al.* (2008) studied the relationship between the evaporation and vapour pressure of volatile chemicals. The objective of this study is to use correlation studies to analyse the quantity and quality of gluten made by varying the three main factors which give large effect to gluten formation, i.e., mixing time, salt and water level using both strong and weak flours. The properties of gluten were measured as four responses of gluten production, i.e., the quantity with respect to its wet and dry gluten contents and its quality in terms of gluten volume expansion from frying (Chen *et al.*, 1998; Chiang *et al.*, 2006) and extensibility from uniaxial extension test.

MATERIALS AND METHODS

The experiments were performed in the Laboratory of Biological and Engineering Materials of the Department of Process and Food Engineering in Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The two types of flour, Diamond N (12.33% protein) and SP-3 (8.81% protein) were referred as the strong and weak flour, respectively. The dough and gluten samples were prepared following methods described by Abang Zaidel *et al.* (2008). Figure 1 presents the flowchart of gluten samples preparation from doughs for their quantity and quality evaluation. The preparation of gluten was divided into three parts which are for the wet and dry gluten, volume expansion and extensibility analyses.

Quantity analysis: For quantity analysis, the dough mixed from 200 g flour was divided into six small doughs weighed based on 25 g flour calculated as Eq. 1:

$$\text{Amount of dough} = \frac{(100\% \text{ flour} + a\% \text{ water} + b\% \text{ salt})}{100} \times 25 \text{ g of flour} \quad (1)$$

where, a and b are the water and salt levels, respectively, used in the flour-water mixing.

The gluten was washed following the AACC Method 38-10, described by Abang Zaidel *et al.* (2008). The rested gluten was removed from the water and pressed dry between cloths, rolled into a ball shape and weighed on a 5×5 cm piece of aluminium foil. It was repeated for all 6 gluten ball samples. The percentage of the wet gluten content was calculated based on 25 g of flour as shown in Eq. 2.

$$\text{Wet gluten (\%)} = \frac{\text{Weight of wet gluten (g)}}{25 \text{ g flour}} \times 100 \quad (2)$$

The dry gluten was obtained by drying the wet gluten in an oven (UM200-800, Memmert GmbH+Co.KG, Germany) to constant weight at 100°C for 24 h, using the air oven drying method. The dried gluten was left to cool for 1 h before taking its weight as the dry gluten content. The percentage of dry gluten obtained was calculated using Eq. 3.

$$\text{Dry gluten (\%)} = \frac{\text{Weight of dry gluten (g)}}{25 \text{ g flour}} \times 100 \quad (3)$$

The average value of the 6 samples was taken for both wet and dry gluten content.

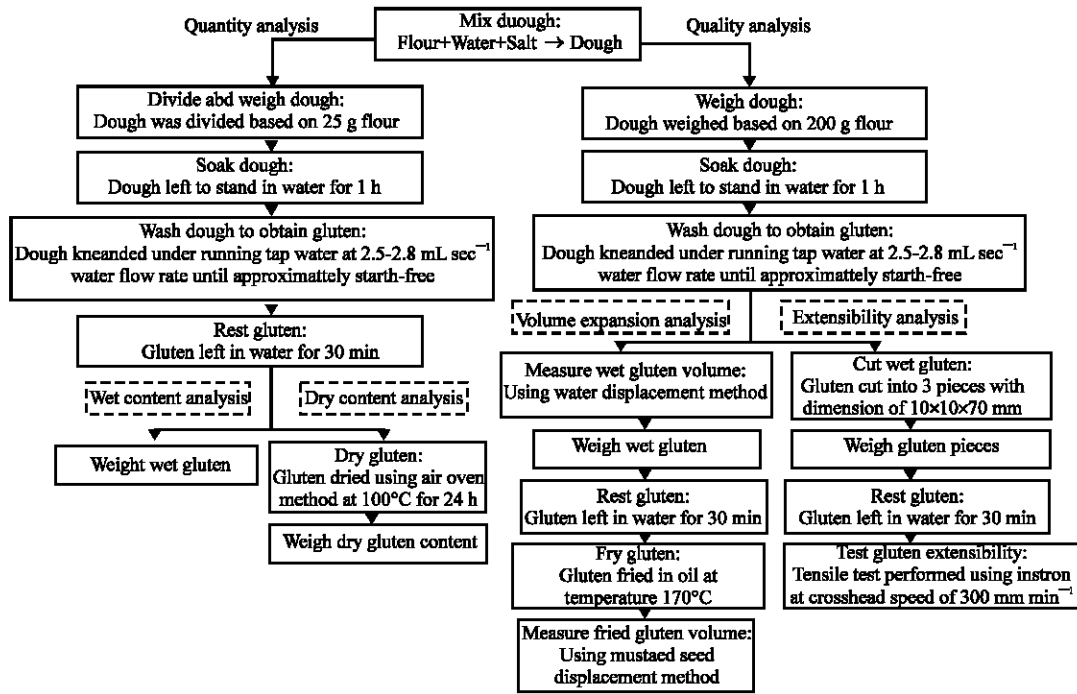


Fig. 1: Methods and preparations of dough and gluten for quantity and quality analyses

Table 1: Alpha, low, center and high points for the experimental design

Factors	-Alpha (-α)	Low point (-1)	Center point (0)	High point (+1)	+Alpha (+α)
Mixing time (min)	3.00	5.00	8.00	11.00	13.00
Salt level (%)	0.00	2.00	5.00	8.00	10.00
Water level (strong) (%)	60.04	61.40	63.40 (O)	65.40	66.76
Water level (weak) (%)	56.10	57.50	59.50 (O)	61.50	62.90

Volume expansion analysis: The volume of wet gluten obtained based on 200 g flour was measured using water displacement method and the volume was recorded as V_1 . The wet gluten was then weighed and immersed in water for 30 min at room temperature for resting (Chen *et al.*, 1998; Chiang *et al.*, 2006). Frying of gluten was performed in a 2l deep-fryer (PDF-9989, Pensonic, Malaysia) at about $170 \pm 5^\circ\text{C}$ for 15 min. The volume of fried gluten was measured abruptly upon removal from the oil and left alone for 5 min for oil draining using the mustard seed displacement method. About 20 mL of mustard seeds were poured into a container with volume (V_1) to fill up its base. The fried gluten was put into the container and more mustard seeds were poured into the container until a slight overflow and the excess seeds were scraped off the top of the container with a ruler. With the aid of a funnel, the seeds used in the container were transferred into a measuring cylinder to obtain its volume, V_2 . The volume of fried gluten (V_f), was calculated as $V_f = V_1 - V_2$. The volume expansion of fried gluten, (V_e), was obtained using $V_e = V_f - V_1$.

Extensibility analysis: The extensibility test was performed following the method of gluten characterisation described in Abang Zaidel *et al.* (2008). Gluten samples obtained from rested doughs were shaped into strips of $10 \times 10 \times 70$ mm, which weighed approximately 5.5 ± 0.5 g, were immersed in tap water at room temperature for 30 min before conducting the extensibility test using a V-shaped hook fitted to the Instron (5566 series, Instron Corporation, USA) to pull the sample at speed of 300 mm min^{-1} until the gluten strip breaks (Chen *et al.*, 1998; Chiang *et al.*, 2006; Abang Zaidel *et al.*, 2008). The extensibility parameter, length of gluten at fracture, (l_f), is reported as derived in Abang Zaidel *et al.* (2008).

Experimental design and analysis: The experiment design was constructed using Response Surface Methodology (RSM) using the Design Expert software with three coded levels for each factor (Table 1). Table 2 and 3 show the experimental design constructed for gluten quantity and quality analysis, respectively. A total of 17 runs with 6 star points ($-\alpha, +\alpha$) and three replicates of center

Table 2: 2³⁻¹ fractional factorial central composite design for quantity analysis

Run	Coded factors		
	Mixing time (min) A	Salt level (%) B	Water level (%) C
1	0	-1.68179	0
2	+1	-1	-1
3	-1	-1	+1
4	0	0	+1.68179
5	-1	+1	-1
6	-1.68179	0	0
7	-1	+1	+1
8	+1	-1	+1
9	0	0	0
10	+1.68179	0	0
11	+1	+1	-1
12	-1	-1	-1
13	0	0	0
14	+1	+1	+1
15	0	+1.68179	0
16	0	0	-1.68179
17	0	0	0

Table 3: 2³⁻¹ fractional factorial central composite design for quality analysis

Run	Coded factors		
	Mixing time (min) A	Salt level (%) B	Water level (%) C
1	0	-1.68179	0
2	+1	+1	-1
3	+1	+1	+1
4	+1	-1	-1
5	+1.68179	0	0
6	0	0	+1.68179
7	-1	+1	-1
8	0	0	0
9	-1	-1	-1
10	-1	-1	+1
11	+1	-1	+1
12	+1	+1	+1
13	-1.68179	0	0
14	-1	+1	+1
15	0	1.68179	0
16	+1	+1	-1
17	-1	+1	-1
18	-1	+1	-1
19	+1	-1	-1
20	-1	-1	+1
21	0	0	0
22	0	0	0
23	-1	+1	+1
24	0	0	0
25	-1	-1	-1
26	0	0	0
27	-1	+1	+1
28	+1	-1	+1
29	+1	+1	+1
30	0	0	0
31	+1	-1	-1
32	0	0	-1.68179
33	-1	-1	+1
34	-1	-1	-1
35	+1	+1	-1
36	+1	-1	+1

points (0) were constructed for experiments of quantity analyses. There was no replicate for factorial points. For the quality analyses, there were a total of 36 runs for each volume expansion and extensibility analysis, with 6 star points (- α , + α), 3 replicates of factorial points (-1, +1) and 6 replicates of center points (0). All reported data were average values of the replicate samples. Simple regression analysis (Ćurić *et al.*, 2001; Xu *et al.*, 2008) was performed using statistical software, MINITAB Release 14 to determine the linear correlation coefficient, R and coefficient of determination, R² for the correlation between two responses of gluten quantity and quality measurements.

RESULTS AND DISCUSSION

The correlation between two analyses of gluten quantity and quality measurements was evaluated by determining the linear correlation coefficient, R and the coefficient of determination, R². The value of R indicates the strength and the direction of a linear correlation between two variables while R² indicates the percent of the data that is the closest to the line of best fit (Ćurić *et al.*, 2001; Xu *et al.*, 2008). Linear correlation coefficient lies within the range -1 < R < +1 where -1 and +1 demonstrate that the two measurements are strongly negatively and positively correlated, respectively. Table 4 and 5 show the values of R and R² for the correlation between two analyses of gluten quantity and quality measurements. The R and R² values gave positive linear correlation for both strong and weak flours. It is clear that strong flour gluten has stronger positive linear correlations (R > 0.60) between two analyses of gluten quantity and quality measurements than weak flour (R > 0.30). From the good correlation between quantity and quality of gluten from strong flour, it is understood that why gluten from strong flour has greater capability in terms of strength, thus suitable for use in breadmaking. This analysis agrees with studies by Janssen *et al.* (1996) and Kokelaar *et al.* (1996) indicated strong positive correlation was obtained for baking quality of bread from flour with higher protein content or the strong flour compared to the weak flour. Janssen *et al.* (1996) assessed breadmaking performance of two different glutes and found that the gluten from high protein content produced higher loaf volume bread compared to the low protein content gluten. A study by Sliwinski *et al.* (2004b) reported that gluten with lower protein content produced smaller bread volume, exhibited lower strain hardening and smaller fracture strain, which demonstrates weak gluten network developed during mixing.

Table 4: Linear correlation coefficient, R, between gluten quantity and quality measurements

R	Strong flour				Weak flour			
	Wet gluten (%)	Dry gluten (%)	V _e (mL)	l _f (mm)	Wet gluten (%)	Dry gluten (%)	V _e (mL)	l _f (mm)
Wet gluten (%)	1.00				1.00			
Dry gluten (%)	0.918***	1.00			0.994***	1.00		
V _e (mL)	0.852***	0.804***	1.00		0.491**	0.509**	1.00	
l _f (mm)	0.784***	0.895***	0.605***	1.00	0.615***	0.603***	0.315*	1.00

Correlation is *significant at p≤0.05; **significant at p≤0.001; ***significant at p≤0.0001

Table 5: Coefficient of determination, R², between gluten quantity and quality measurements

R ²	Strong flour				Weak flour			
	Wet gluten (%)	Dry gluten (%)	V _e (mL)	l _f (mm)	Wet gluten (%)	Dry gluten (%)	V _e (mL)	l _f (mm)
Wet gluten (%)	1.00				1.00			
Dry gluten (%)	0.844	1.00			0.987	1.00		
V _e (ml)	0.725	0.647	1.00		0.241	0.259	1.00	
l _f (mm)	0.615	0.802	0.366	1.00	0.378	0.363	0.0995	1.00

Table 6: Results of wet and dry gluten content, volume expansion of fried gluten and gluten length at fracture at optimum water level (63.40% water level for strong and 59.50% for weak flour) for various mixing times and salt levels

Salt (%)	Mixing time (min)	Strong flour				Weak flour			
		Wet gluten (%)	Dry gluten (%)	V _e (mL)	l _f (mm)	Wet gluten (%)	Dry gluten (%)	V _e (mL)	l _f (mm)
2	3	37.82	14.19	469	251	27.00	9.70	224	165
	5	38.20	14.30	475	255	27.20	9.70	260	173
	8	38.70	14.40	500	260	27.40	9.80	285	177
	11	39.30	14.50	515	258	27.60	9.90	280	165
	13	39.74	14.62	520	255	27.90	9.90	262	153
5	3	35.87	12.72	300	175	25.70	9.30	130	109
	5	36.00	12.90	385	210	25.60	9.20	260	148
	8	36.59	13.00	385	221	25.50	9.30	294	151
	11	36.30	13.20	365	220	25.40	9.20	285	145
	13	38.30	14.12	440	246	25.30	9.20	200	105
8	3	33.99	11.51	372	156	23.00	8.10	156	121
	5	33.60	11.60	365	170	22.80	8.10	190	134
	8	33.20	11.70	325	180	22.60	8.00	220	148
	11	33.00	11.80	300	190	22.40	7.90	200	134
	13	32.92	11.94	282	194	22.00	7.90	195	120

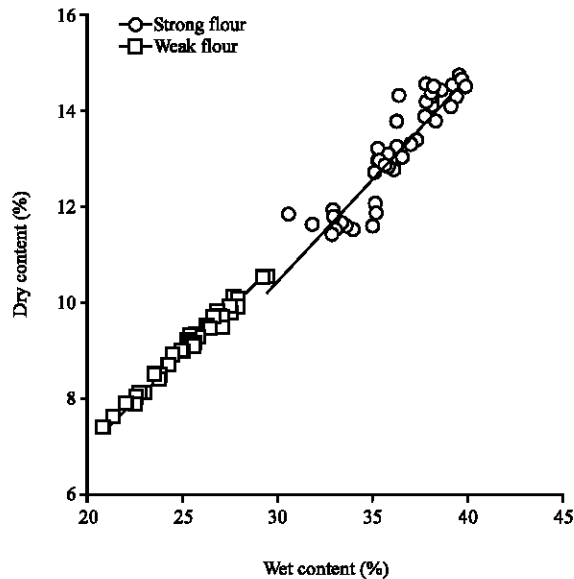


Fig. 2: Correlation between dry and wet gluten content for strong (R = 0.918) and weak (R = 0.994) flour

Figure 2 shows a strong positive correlations between dry and wet gluten content (R = 0.918 for strong flour; R = 0.994 for weak flour). This is highly due to water evaporation during the process of air oven drying (Georgopoulos *et al.*, 2004) to obtain the dry content of gluten. Table 6 shows the details on each results on wet and dry gluten content, volume expansion of fried gluten and gluten length at fracture at optimum water level (63.40% water level for strong and 59.50% for weak flour) for various mixing times and salt levels. A close observation suggests that the wet and dry gluten amount for both flours seem to decrease as salt levels increased. This could be resulted by the removal of globulin during gluten washing due to its high solubility in salt solution (Kuktaitė, 2004) and thus reduced the quantity of gluten remained from dough washing.

Figure 3 shows the correlation between volume expansion and wet gluten content for the strong and weak flour gluten. Positive linear correlations were observed for both flours demonstrating an increase in the volume expansion of fried gluten as the wet gluten content

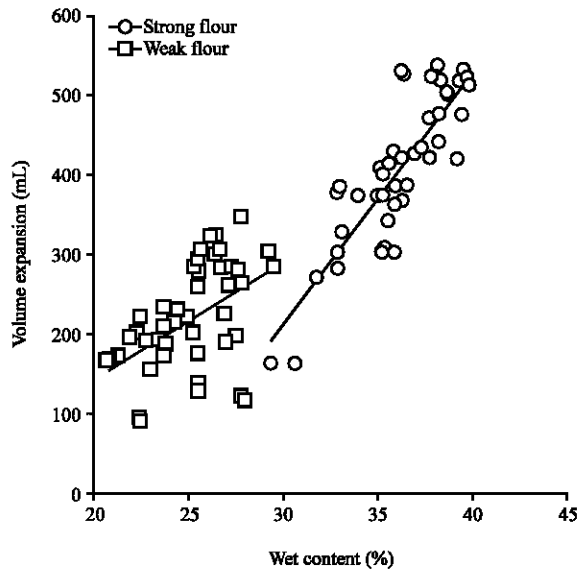


Fig. 3: Correlation between volume expansion and wet gluten content for strong ($R = 0.852$) and weak ($R = 0.491$) flour

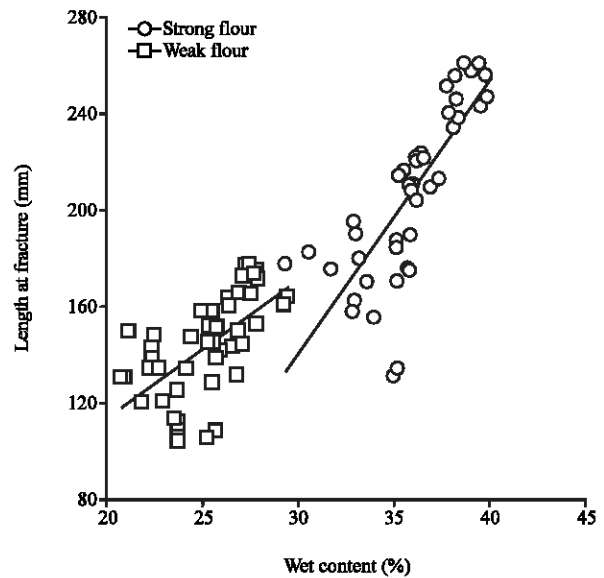


Fig. 5: Correlation between gluten length at fracture and wet gluten content for strong ($R = 0.784$) and weak ($R = 0.615$) flour

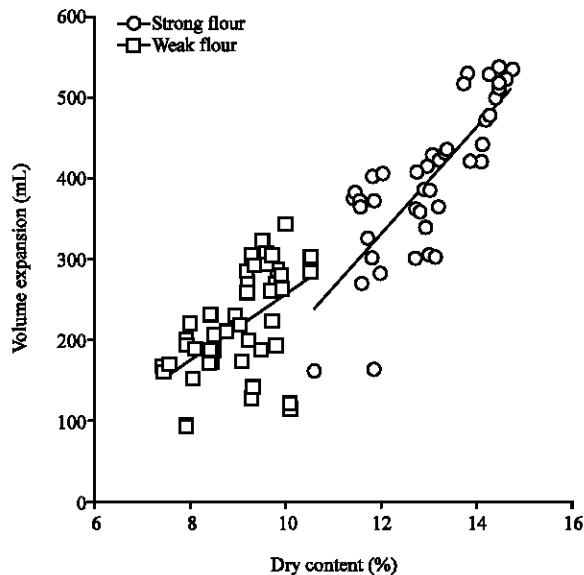


Fig. 4: Correlation between volume expansion and dry gluten content for strong ($R = 0.804$) and weak ($R = 0.509$) flour

increased. Stronger positive linear correlation was observed for strong flour ($R = 0.852$) compared to the weak flour ($R = 0.491$). The quantity of protein in flour which usually dictates its strength has an effect on the volume expansion of the fried gluten as reported in Chiang *et al.* (2006). This is also why flour with higher gluten content produces higher quality of breads i.e.,

having bigger loaf volume and greater extensibility Sliwinski *et al.* (2004a). Few other researchers also presented that protein content of the flour is directly related to the strength of the gluten network produced from mixed dough (Tronsmo *et al.*, 2003; Sliwinski *et al.*, 2004c). In Fig. 4, the volume expansion of fried gluten and dry gluten content for both flours have a positive linear correlation ($R = 0.804$ for strong flour; $R = 0.509$ for weak flour). A stronger correlation between the volume expansion and dry gluten content in the strong flour is explained by the higher protein and dry gluten contents in strong flour. An interesting finding is that as salt levels increased from 2 to 8%, the gluten for both strong and weak flour showed a decrease in volume expansion (V_v) (Table 6). The presence of salt reduces the elasticity of the gluten network (Salvador *et al.*, 2006) thus reducing the volume of gluten expanded during frying. Besides the presence of salt, factors like mixing time and water levels also affected the volume expansion of fried gluten. During dough mixing, the gluten network is developed as mixing time increases and the interactions between the polymer cross-links are becoming stronger which leads to an increase in gluten strength (Létang *et al.*, 1999).

The correlation between gluten length at fracture and wet gluten content for both strong and weak flour is shown in Fig. 5. Both flours show positive linear correlation, with the strong flour giving a slightly stronger positive linear correlation ($R = 0.784$) than the weak flour ($R = 0.615$). This again suggests that the protein

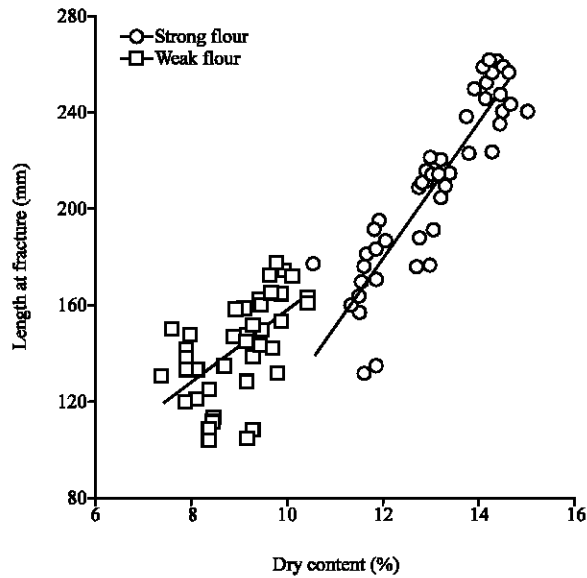


Fig. 6: Correlation between gluten length at fracture and dry gluten for strong ($R = 0.895$) and weak ($R = 0.603$) flour

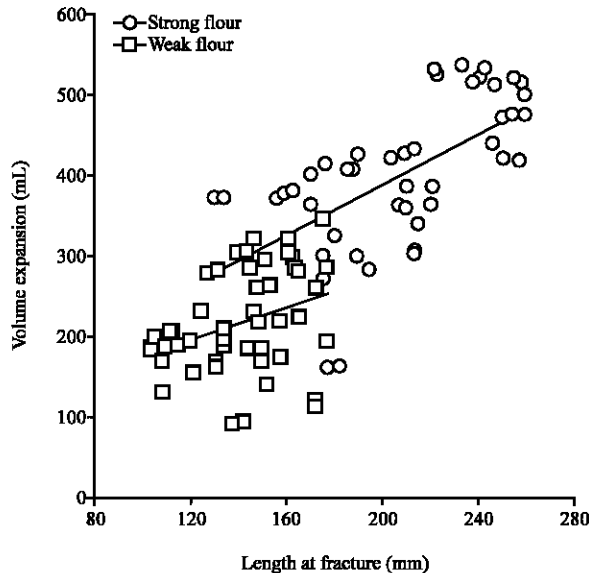


Fig. 7: Correlation between volume expansion and gluten length at fracture for strong ($R = 0.605$) and weak ($R = 0.315$) flour

content of the flour determines the strength of gluten (Tronsmo *et al.*, 2003; Sliwinski *et al.*, 2004c). The correlation between the gluten length at fracture and dry gluten content is presented in Fig. 6 ($R = 0.895$ for strong flour; $R = 0.603$ for weak flour). Similarly, as the dry gluten content increased, the maximum length at fracture increased. Salt has a same effect on gluten extensibility

i.e., decreases gluten extensibility as it has to the gluten contents and volume expansion. The extensibility for both strong and weak flour increased with mixing time (Table 6) although an optimum was observed for the weak flour at a mixing time of 8 min. Similar explanation is used for this observation. The building of gluten network as mixing time increases and the stronger interactions between the polymers cross-links lead to an increase in gluten strength and extensibility. When mixing passes its optimum, the mixing action will break the gluten cross-links due to the breaking of disulphide bonds resulting gluten to become weaker and lower in extensibility (Létang *et al.*, 1999).

Of all correlations analysed, Fig. 7 shows the weakest correlation in the volume expansion of fried gluten and the gluten length at fracture despite the strong flour having a stronger positive linear correlation ($R = 0.605$) compared to weak flour ($R = 0.315$). Chiang *et al.* (2006) also reported that gluten expansion from strong flour resulted from frying is strongly affected by its extensibility and that the correlation between the gluten volume expansion with extensibility is weaker for flour with lower protein contents.

CONCLUSION

This study uses a systematic approach towards evaluating the physical behavior of gluten. It quantifies the correlation between gluten quantity measured as its wet and dry gluten contents and gluten quality measured as gluten volume expansion and extensibility. These analyses provide a clearer picture of gluten properties and behavior besides giving additional support to existing literatures reporting the relationships and correlations between dough extensibility and bread loaf volume and the effect of protein content on flour strength. All correlations between the two analyses of gluten quantity and quality measurements gave positive linear correlation coefficient, R . It shows that as the quantity of the gluten increases, the quality of gluten increases. The volume expansion of fried gluten increases as the extensibility of gluten increases. Stronger correlation were obtained for strong flour for all correlation studies, between (i) gluten quantity and volume expansion (ii) gluten quantity and extensibility and (iii) volume expansion and gluten extensibility i.e., ($R > 0.75$; $R > 0.80$; $R > 0.60$) compared to weak flour ($R > 0.45$; $R > 0.50$; $R > 0.30$), respectively.

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NOMENCLATURE

- l_f : Gluten length at fracture mm
- R : Linear correlation coefficient
- R^2 : Coefficient of determination
- V_1 : Volume of container (mm^3)
- V_2 : Volume of displaced seeds (mm^3)
- V_i : Volume of wet gluten (mm^3)
- V_e : Volume expansion of gluten (mm^3)
- V_f : Volume of fried gluten (mm^3)

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