Title: Effect of water content and flour particle size on gluten-free bread quality and digestibility

Authors: Esther de la Hera[a,b], Cristina M. Rosell[b], Manuel Gomez[a*]

Affiliations: [a]Food Technology Area, E.T.S. Ingenierías Agrarias. University of Valladolid, Edificio La Yutera, Avenida Madrid, 44, 34004, Palencia, Spain. [b]Institute of Agrochemistry and Food Technology (IATA-CSIC). Avenida Agustin Escardino, 7. Paterna 46980. Valencia. Spain.

Address for correspondence: Manuel Gómez

Tel +34 979108495, Fax: +34 979108302, e-mail: pallares@iaf.uva.es

Abstract

The impact of dough hydration level and particle size distribution of the rice flour on the gluten free bread quality and *in vitro* starch hydrolysis was studied. Rice flour was fractionated in fine and coarse parts and mixed with different amounts of water (70%, 90% and 110% hydration levels) and the rest of ingredients used for making gluten free bread. Larger bread specific volume was obtained when coarser fraction and great dough hydration (90-110%) were combined. The crumb texture improved when increasing dough hydration, although that effect was more pronounced when breads were obtained from fine fraction. Estimated glycaemic index was higher in breads with higher hydration (90-110%). Slowly digestible starch (SDS) and resistant starch (RS) increased in coarse flour breads. Coarse fraction complemented with great dough hydration (90-110%) was the most suitable combination for developing rice bread when considering bread volume and crumb texture. However, the lowest dough hydration limited starch gelatinization and hindered the *in vitro* starch digestibility.

Keywords: Gluten-free bread; particle size; water content; starch digestibility

1. Introduction

Celiac disease (CD) has become an increasingly recognized autoimmune enteropathy triggered by the ingestion of some cereal prolamines. In Europe, the prevalence of CD is between 0.3 and 2%, depending on the geographic area evaluated (Mustalahti et al., 2010). Along with genetic susceptibility, environmental factors may play a role in the development of celiac disease (Niewinski, 2008), also timing of the introduction of gluten in infancy was demonstrated to be an important factor (Norris et al., 2005). The individual's intolerance to gluten is lifelong and self-perpetuating, being the only treatment a strict adherence to gluten-free diet (GFD). Despite the benefits of a GFD on symptoms, numerous negative sequelae have been reported: lower intakes of essential micronutrients, vitamins and minerals and higher intakes of sugar (Wild, Robins, Burley & Howdle, 2010).

Given the changes in diet and in the small intestinal absorptive function following the gluten-free diet treatment, significant changes in body mass index may be expected (Dickey & Keame, 2006; Ukkola et al., 2012). Moreover, CD is usually related to associate diseases such as anaemia and type I diabetes. Nevertheless, type I diabetes is diagnosed first than CD in the 90% of the cases (Holmes, 2001). Since celiac disease is associated with a high incidence of type I diabetes (Cronin & Shanahan, 1997), they should maintain good glycaemic control whilst adhering to a strict gluten-free diet.

The glycaemic index (GI) defined as "the area under curve of blood glucose after eating a food containing a determined quantity of carbohydrate" provides an indirect measure of the ability of a food to raise blood glucose and a direct one of the absorption of carbohydrates. The glycaemic index classification of foods has been used as a tool to assess prevention strategies for diseases where glycaemic control plays an important role, such as obesity and diabetes. So far, celiac patients were advised only to avoid gluten in their diet but taking into account nutritional quality of gluten-free products left unsaid. On this line, Esfahani, Wong, Mirrahimi, Srichaikul, Jenkins and Kendall (2009) compiled some studies showing a significant protective effect against the risk of developing diabetes with the lowest dietary glycaemic index intake.

Enzymatic digestion of starch can be affected by many factors such as granule structure, the presence of lipids, proteins or minerals, amylose:amylopectin ratio, digestion conditions and particle size (Al-Rabadi, Gilbert & Gidley (2009). The presence of proteins or lipids influences starch digestion reducing glycaemic response by limiting starch accessibility encapsulating it (Fardet, Leenhardt, Lioger, Scalbert & Rémésy, 2006). And the effect of particle size is usually related to the surface area available for enzymatic action. In this regard, Blasel, Hoffman and Shaver (2006) found the degree of starch access by α-amylase to decrease by 26.8g/kg starch for each 100µm increase in particle size in ground corn grain. Regarding bread, Fardet et al. (2006), studying gluten containing breads, considered the physical structure as the most important factor influencing GI, stating that the more compact the structure, the lower the glycaemic response. Nevertheless, there is no information about how that could be beneficial when obtaining gluten free breads, and neither if by controlling process conditions or raw materials is possible to modulate the glycaemic index, and therefore the starch hydrolysis, of the gluten free bread.

The aim of this study was to assess the effect of particle size of rice flour (fine and coarse) and dough hydration , one of the most critical parameters in gluten free breadmaking, on the physical quality and starch enzymatic digestion of gluten-free breads.

2. Materials and methods

2.1 Materials

Commercial rice flour supplied by Harinera Castellana (Medina del Campo, Spain) had moisture and protein of 12.19 g/100g and 7.22 g/100g, respectively. Salt, sugar, and sunflower oil were purchased from the local market. Dry yeast (Saf-instant, Lesaffre, Lille, France) and hydroxypropyl methylcellulose (HPMC) (Methocel K4M, Dow Chemical, USA) were used.

2.2 Flour Obtaining Process

Flour was sifted in a Bühler MLI 300B (Bühler AG, Uzwil, Switzerland) with screens of 132 and 200 µm to obtain fine and coarse fractions. The so-called fine flour had particle size lower than 132µm, and the coarse fraction contained particles which size ranged between 132µm and 200µm. Those particle sizes were selected based on authors' previous research (de la Hera, Talegon, Caballero & Gomez, 2012) conducted with corn, that pictured the influence of the particle size of corn flour on gluten free bread performance concluding that coarser flours (>180 µm) provide breads with higher volume and softer crumbs.

Fine and coarse flours were used as raw material for gluten free bread making. Since flour hydration is crucial for gluten free breadmaking performance (Marco & Rosell, 2008), three different hydrations were applied to determine whether they could affect starch features and consequently glycaemic index of the resulting bread.

2.3 Bread Making Process

A straight dough process was performed using a Kitchen-Aid Professional mixer (KPM5, KitchenAid, St. Joseph, MI, USA) with a dough hook (K45DH). The following ingredients (as % on wet flour basis) were used: sunflower oil (6%), sucrose (5%), salt (1.8%), dry yeast (3%), HPMC (2%) and water (70, 90 or 110%). Water content or dough hydration was referred to the amount of water used in each recipe. All ingredients were mixed during 8 minutes at speed 2 (in a scale 1-10 of the mixer). Dough pieces (250g) were placed into aluminium pans (232 x 108 x 43.5 mm) and fermented in a proofing chamber at 30ºC and 90% relative humidity for 60 minutes. After proofing, dough was baked in an electric oven for 40 minutes at 200ºC. Then loaves were removed from the pans, cooled for 50 minutes at room temperature, and packed in sealed polyethylene bags to prevent dehydration. Analytical measurements were made within 24 hours. Two batches were made for each sample.

2.4 Analytical Methods

Flour protein content was determined following AACC method 46-30, performed with a Leco TruSpec®N nitrogen/protein analyser.

Bread moisture content was determined following AACC method 44-01.01 (AACC, 2000). Weight loss during baking was assessed by weighing the pans before and after baking. Bread volume was determined using a laser sensor with the BVM-L 370 volume analyzer (TexVol Instruments, Viken, Sweden). The bread specific volume was calculated as the ratio between the volume of the bread and its weight. These measurements were carried out in three breads of each batch.

Crumb texture was determined using a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) with the ‘‘Texture Expert’’ software. A 25 mm diameter cylindrical aluminium probe was used in a ‘Texture Profile Analysis’ (TPA) double compression test to penetrate to 50% depth, with a test speed of 2 mm/s, and a 30-second delay between the first and second compressions. Hardness, cohesiveness, springiness and resilience were calculated from the TPA plot. Measurements were made on two central slices (20 mm thickness) from three breads of each batch.

2.5 *In vitro* Starch Digestibility and Estimated Glycaemic Index

Two slices were freeze dried for determining *in vitro* digestibility. Enzymatic hydrolysis of gluten-free bread was determined following the method reported by Gularte and Rosell (2011) using 100 mg of powdered freeze dried breads. According to the hydrolysis rate of starch, three different fractions were quantified as suggested Englyst, Veenstra and Hudson (1996). Rapidly digestible starch (RDS) was referred to the percentage of total starch that was hydrolyzed within 30 min of incubation, slowly digestible starch (SDS) was the percentage of total starch hydrolyzed within 30 and 120 min, and resistant starch (RS) was the remnant starch after 16 h of incubation. The percentage of total starch hydrolyzed at 90 min (H90) was also calculated.

The *in vitro* digestion kinetic was calculated in accordance with the procedure established by Goñi, Garcia-Alonso and Saura-Calixto (1997). A nonlinear model following the equation [$C = C_\infty (1-e^{-kt})$] was applied to describe the kinetic of starch hydrolysis, where C was the concentration at *t* time, C_∞ was the equilibrium concentration or maximum hydrolysis extent and *k* was the kinetic constant. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (0–180 min) of the sample by the area of a standard material (white bread) over the same period of time. The estimated glycaemic index (eGI) was calculated using the equation described by Granfeldt et al. (1992): eGI = 8.198 + 0.862HI.

2.6 Statistical Analysis

Data were subjected to a two-way analysis of variance (ANOVA) to study the differences in bread quality induced by particle size and dough hydration. A one-way ANOVA was carried out for analysing texture parameters of breads individually, that analysis being necessary when missing experimental data. Fisher's least significant difference (LSD) test was used to describe means with 95% confidence. A correlation analysis was also carried out to determine possible relationships among parameters. Statgraphics Plus Centurion XVI (Statpoint Technologies, Warrenton, USA) was used as statistical analysis software.

3. Results and Discussion

3.1 Physical characteristics of gluten-free breads

Breads obtained from the two different rice flour fractions were physically characterized (Table 1, Figure 1). The specific volume significantly increased when coarse flour was used to obtain breads, and also a steady increase of specific volume was also observed when enhancing water content of dough. The fact that coarse flour yields better loaf specific volume was also observed by de la Hera et al. (2012) in corn bread made with different particle size fractions. It seems that small particles form a weak dough structure, which is probably unable to retain the gas released during fermentation, yielding lower volumes (de la Hera et al., 2013). In addition, de la Hera et al. (2013) found no significant relationship between the particle size of the fractions and the level of damage starch. Therefore, results obtained with the different flour fractions could not be attributed to possible differences in the level of damage starch. The impact of water content was readily evident in the volume of breads obtained from fine flour (Figure 1). The plasticizer effect of the water is crucial when making gluten-free breads because it contributes to the extensional properties of the dough during mixing (Marco & Rosell, 2008). It must be stressed that fine particles increase the contact surface, thus greater amount of water is necessary for hydrating the raw material and later on for swelling starch granules. This could be the explanation of the very low specific volume achieved by breads obtained from fine flour and low water content (70%). Nevertheless, that deficiency was partially corrected when the amount of water increased allowing the hydration of the particles (Table 1). Han, Cho, Kang and Koh (2012) reported that excessive water caused overexpansion during baking resulting in large-volume breads. Nevertheless, too much water led big holes in the crumb (Figure 1), and that effect was more visible in coarse flour containing breads. The combined effect of flour particle size and recipe water content can be observed in the two-way ANOVA interaction graphic (Figure 2). Plots in figure 2 indicated that differences in bread specific volume derived from diverse particle size can be minimized increasing the water content. Specific volume of breads made with coarse flour and 90 or 110% of water did not show significant differences, suggesting that optimal water content for this particle size is around 90-110%.

Coarse flour led to breads with lower water retention ability as indicated its significantly higher weight loss, which also increased with higher water content (Table 1). The lower water retention might be attributed to the lower hydration ability of the coarse particles compared to the fine ones. In fact, de la Hera, Gómez and Rosell (2013) reported that behaviour and it was explained by the lower surface area of large particles in comparison with small ones. A significant positive correlation was obtained between the weight loss and the specific volume (r=0.7440; $P\leq 0.05$), which indicated that the greatest the surface in contact with air inside the oven the highest water evaporation during baking and thus high weight loss.

Crumb texture parameters (Table 2) were measured to assess the bread quality. Bread made with fine flour and 70% of water content could not be assessed because its volume was not high enough to allow compression with full contact between crumb and probe surface. Hardness decreased significantly in breads made with coarse flour compared to the ones obtained from fine flour. A reduction of the hardness was observed when increasing the water content in the recipe, although no significant differences were detected between water content of 90% and 110% in breads made with coarse flour. Hardness was inversely correlated with specific volume (r=-0.8931; $P\leq 0.001$), thus lower bread specific volume results in greater hardness due to denser crumb and more compact cells. Crumb hardness of the rice breads obtained in this study was much lower than the one reported for commercial gluten free breads (Matos & Rosell, 2012), probably due to the use of starch instead of flour.

Cohesiveness, which quantifies the internal resistance or cohesion of food structure, significantly increased in breads made with fine flour. The effect of water was only significant with 70 and 90% hydration, decreasing cohesiveness in parallel to the increase of water content in formulation. In fact, cohesiveness showed high significant correlation with bread moisture content (r=0.9008; $P\leq0.001$). Low cohesiveness indicates high susceptibility of the crumb to fracture or crumble. Considering volume results, besides hardness and cohesiveness, it seems that in breads obtained from 70% dough hydration the limited amount of water impeded intermolecular interaction among ingredients and prompted water competition among ingredients (Parada & Aguilera, 2011). Conversely, springiness, indicative besides resilience of the crumb elasticity, was affected by water content in breads made with coarse flour. In those breads, the highest water content led to the highest springiness. High resilience values were observed in breads made of fine flour, in which water content enhancement significantly increased resilience values. The water content effect on resilience in breads made of coarse flour was only observed when 70% or 90% hydration was applied. Springiness and resilience are commonly related and their reduction has been related to loss of crumb elasticity (Onyango, Mutungi, Unbehend & Lindhauer, 2011). However, considering overall texture results, it seems that in rice based gluten free breads it is advisable to loss some extent of the elasticity in favour of softness and cohesiveness.

3.2 Starch digestibility in gluten-free breads

Starchy foods, like bread, result in rapid degradation in the small intestine due to almost all starch is gelatinized (Parada & Aguilera, 2011). Results agree with this pattern, being RDS the most predominant starch fraction in all breads and varying from 82.07/100g to 96.54g/100g (Figure 3). These values falls within the ones previously reported for commercial gluten free breads (Matos & Rosell, 2011). In general, breads made with coarse flour showed lower values of RDS than those with fine flour, excepting bread with 70% of water. Fine flour mixed with the lowest water content offered very limited water for starch gelatinization, which could be the reason of the scarce hydrolysis in the earliest time of analysis. Slowly digestible starch values ranged from 0.60/100g to 11.40g/100g. SDS is slowly digested in small intestine inducing gradual increase of postprandial plasma glucose and insulin levels (Englyst & Hudson, 1996). The term resistant starch (RS) refers to the sum of intact starch and retrograded starch that pass into the large intestine, which makes the distinction between starch that is hydrolysed and absorbed in the human small intestine (the sum of RDS and SDS), and starch that reaches the human large intestine (RS) (Englyst & Cummings, 1990). In this study, resistant starch varied from 0.89/100g up to 1.96g/100g. Discarding bread made with fine flour and 70% of water, which could hardly be considered bread (Figure 1), SDS and RS were greater in breads from coarse rice flour. In the case of bread made with fine flour seems that the degree of starch gelatinization determined the amount of the different starch fractions. Because of that the higher amount of water the greater RDS fraction is, until the amount of water is no longer limiting. After being gelatinized and thus in disentangled structure, starch granules are readily accessible to enzymes attack, whereas native starch, ungelatinized starch and retrograded amylose are not susceptible to undergo enzymatic hydrolysis. The pancreatic α-amylase affinity for digesting starches is dependent on the degree of order of starch that has important influence on the initial rate at which native starch is digested by amylase (Tahir, Ellis & Butterworth, 2010). Nevertheless, in the case of breads made with coarse flour, it seems that the surface area of the granules plays an important role. De la Hera, Gomez and Rosell (2013) reported differences among enzymatic hydrolysis plots of flour fractions when particle size was higher than 150 µm, displaying slower hydrolysis when increasing the particle size. Tester and Karkalas (2006) described that the larger granules the smaller is the surface area to volume ratio and hence the potential surface to be attacked and hydrolyzed by enzymes. It has been reported that alfa-amylase affinity for native starches is dependent on the particle size of starch, due to the enzyme feasibility for binding/absorption (Tahir, Ellis & Butterworth, 2010). The first step of the enzymatic hydrolysis is the enzyme binding and absorption which will be limited due to the lower surface area compared to the fine flour. As particle size increases, the surface area exposed to digestive enzymes decreases, leading to decreased rate of digestion (Pi-Sunyer, 2002). When assessing the starch granules susceptibility to enzyme hydrolysis is reported that the surface area of granules and the degree of order of starch have important influences on the initial rate at which native starch is digested by amylase (Tahir, Ellis & Butterworth, 2010). In this study, it seems that the enzymatic hydrolysis of the starch in breads made with fine flour is governed by the degree of order of starch, whereas in the case of breads made with coarse flour, the surface area of the particles determines the enzymatic attack.

3.3 Kinetic of the in vitro starch hydrolysis and expected glycaemic index

Parameters derived from the *in vitro* digestion of the gluten-free breads are listed in Table 3. There is a lack of information about starch digestibility and glycaemic response of gluten-free foods; although some authors have reported that the GI of gluten-free bread is significantly higher than that of traditional bread (Berti, Riso, Monti & Porrini, 2004; Matos & Rosell, 2011). The maximum hydrolysis (C_∞), or the hydrolysis degree when the enzymatic reaction showed minimal variation due to the particle size of the flour and the water content used in the recipe. Those high values agree with Jenkins, Thorne, Wolever, Jenkins, Venketschwer and Thomson

(1987) theory about gluten hindering the access of the amylase to starch granules. Those authors suggested that the glycaemic response of carbohydrates might increase following the removal of gluten, because gluten protein network surrounds the starch granules limits the amylase accessibility to the starch granule and in consequence the presence of gluten might slow down the rate of starch hydrolysis. The kinetic constant (k) values, indicative of the hydrolysis rate, were comprised between 0.07 min^{-1} and 0.16 min^{-1}. k was higher for the bread made with fine flour. AUC of gluten free breads was not influenced by particle size of rice-flour but it was significantly affected by the water content. AUC of the breads made with 90% and 110% water content showed higher AUC than the one made with 70% water content. That trend agrees with results of RDS and the same was observed with commercial gluten free breads (Matos & Rosell, 2011). Values of estimated glycaemic index (eGI) ranged from 87 to 93, and although particle size did not affect that index, the water content added for dough making did. AUC, HI, H90 and eGI show the same trend as the kinetic constant regarding water content, which might indicate the greatest influence of k during starch hydrolysis. Breads made with 70% of water content showed the lowest eGI values than the other hydrations tested, what could be explained by the amorphous starch regions that remain part of the starch granular structure and the limited gelatinization of starch granules, which are less prone to be attacked by alfa amylase, as occurred in wheat bread (Roder et al., 2009). Other plausible explanation is that these breads show lower glycaemic response due to their more compact physical structure, which is more preponderant than other parameters governing GI, as Fardet et al. (2006) pointed out.

4. Conclusions

Particle size of raw material, besides dough hydration, plays a significant role in determining gluten free bread quality and *in vitro* starch digestibility. Results of this study indicated that coarse fraction complemented with great dough hydration (90-110%) is the most suitable combination for developing rice bread when considering bread volume and crumb texture. However, regarding nutritional aspects, best combination would be the lowest dough hydration meaning lower volume and greater hardness of bread. Reduction of dough hydration limited starch gelatinization and hindered the *in vitro* starch digestibility and the higher the particle size the greater amount of SDS and RS. Overall this study indicated that particle size and especially dough hydration should be taken into account for modulating the enzymatic hydrolysis of gluten free starchy foods.

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Figure Captions

Figure. 1 Bread central slice cross section. Bread made with fine rice flour (particle size <132µm) (**A**) and coarse flour (particle size 132-200µm) (**B**). Water content of 70% (**a**), 90% (**b**) and 110% (**c**) was added.

Figure 2. Two-way ANOVA interaction graphic between flour particle size and water content on specific volume. Coarse refers to particle size range of 132-200µm and fine refers to particle size under 132µm. Different letters in each point indicate significant differences ($P \leq 0.05$)

Figure 3. *In vitro* starch digestibility in gluten-free breads determined by enzymatic hydrolysis. RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch. Coarse refers to particle size range of 132-200µm and fine refers to particle size under 132µm. Numbers described the water content added in recipe. Letters within each starch fraction are referred to differences from statistical analysis ($P<0.05$).

Table 1. Effect of flour particle size and water content of recipe on some characteristics of rice based gluten free breads.

		Moisture (%)	Specific Volume (mL/g)	Weight Loss (g)
	Overall Mean	37.81	4.17	4.63
Particle Size	Fine	38.41 b	2.64 a	2.86 a
	Coarse	37.21 a	5.71 b	6.40 b
Water Content (%)	70	32.37 a	3.06 a	1.68 a
	90	39.22 b	4.22 b	4.94 b
	110	41.83 c	5.24 c	7.28 c

Values followed by different letters in each column and each parameter indicate significant differences (*P*≤0.05)

Table 2. Textural parameters of bread crumb analyzed trough a one-way ANOVA.

Flour type	Water content (%)	Hardness (N)	Cohesiveness	Springiness	Resilience
Fine	70	---	---	---	---
	90	9.51 ±0.06 c	0.45 ±0.01 cd	0.84 ±0.02 b	0.22 ±0.01 c
	110	1.12 ±0.06 b	0.53 ±0.02 d	0.86 ±0.01 b	0.32 ±0.02 d
Coarse	70	1.76 ±0.58 c	0.23 ±0.01 a	0.54 ±0.04 a	0.09 ±0.01 a
	90	0.61 ±0.08 a	0.34 ±0.04 b	0.54 ±0.04 a	0.15 ±0.04 b
	110	0.80 ±0.07 ab	0.41 ±0.10 bc	0.81 ±0.01 b	0.16 ±0.00 b

Values followed by different letters in each column indicate significant differences (*P*≤0.05)

Table 3. Kinetic parameters of the *in vitro* starch hydrolysis and estimated glycaemic index

		C_∞ (g/100g)	k (min^{-1})	AUC	H90	HI	eGI
	Overall Mean	96.55	0.108	48.82	95	96	91
Particle Size	Fine	97.4 a	0.131 b	48.61 a	95 a	95 a	90 a
	Coarse	95.7 a	0.086 a	49.01 a	96 a	96 a	91 a
Water Content (%)	70	96.5 a	0.085 a	46.99 a	93 a	92 a	87 a
	90	96.4 a	0.120 b	49.41 b	96 b	97 b	92 b
	110	96.7 a	0.120 b	50.05 b	97 b	98 b	93 b

Values followed by different letters in each column and each parameter indicate significant differences ($P \leq 0.05$)

C_∞, equilibrium concentration; k, kinetic constant; HI, hydrolysis index; AUC 180, area under curve; eGI, estimated glycaemic index

Figure 1.

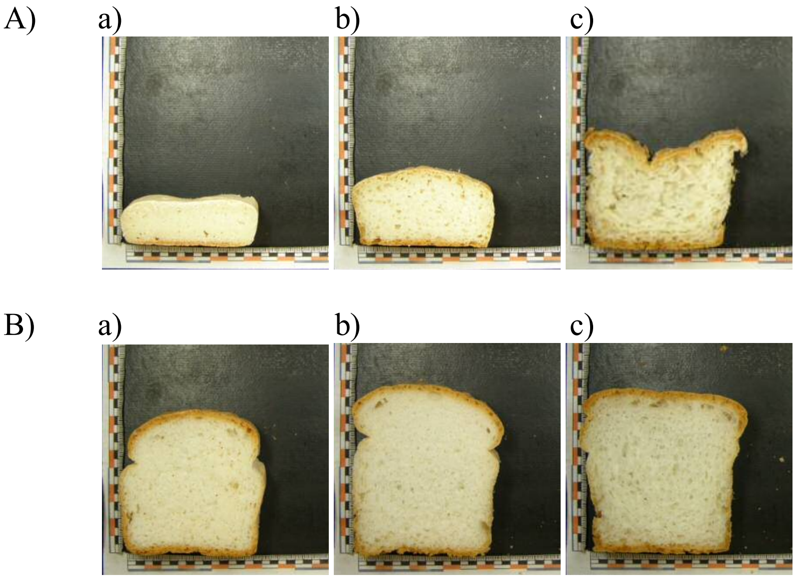


Figure 2.

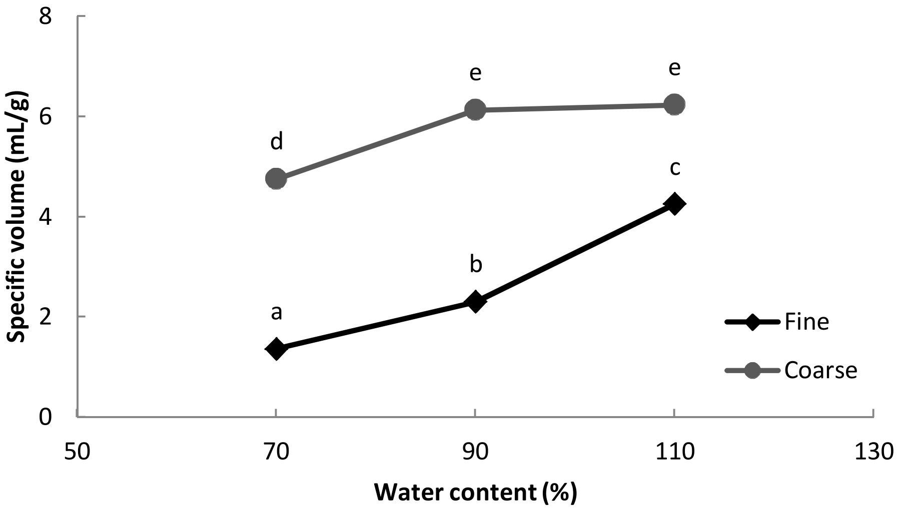
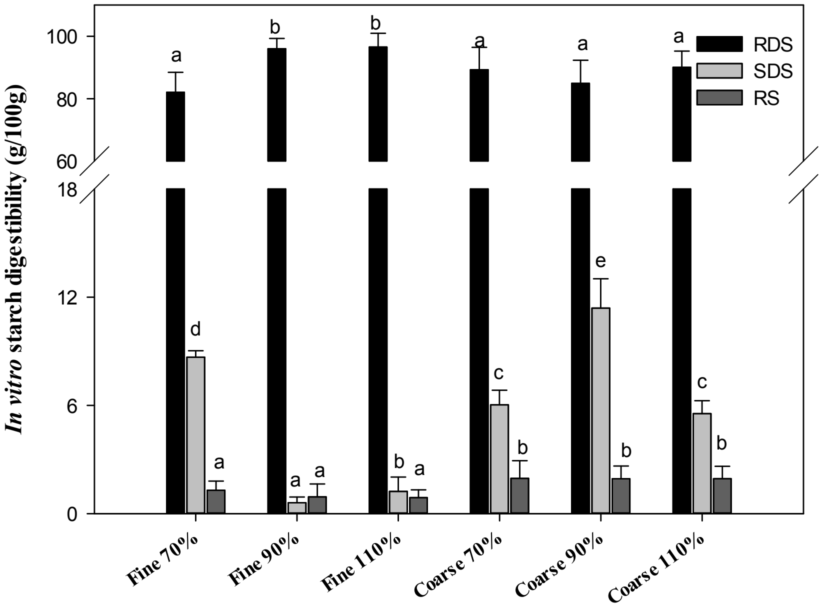


Figure 3.


In vitro starch digestibility (g/100g)
100
80
60
18
12
6
0
RDS
SDS
RS
a
b
b
a
a
a
d
a
a
b
a
a
c
b
e
b
c
b
Fine 70%
Fine 90%
Fine 110%
Coarse 70%
Coarse 90%
Coarse 110%