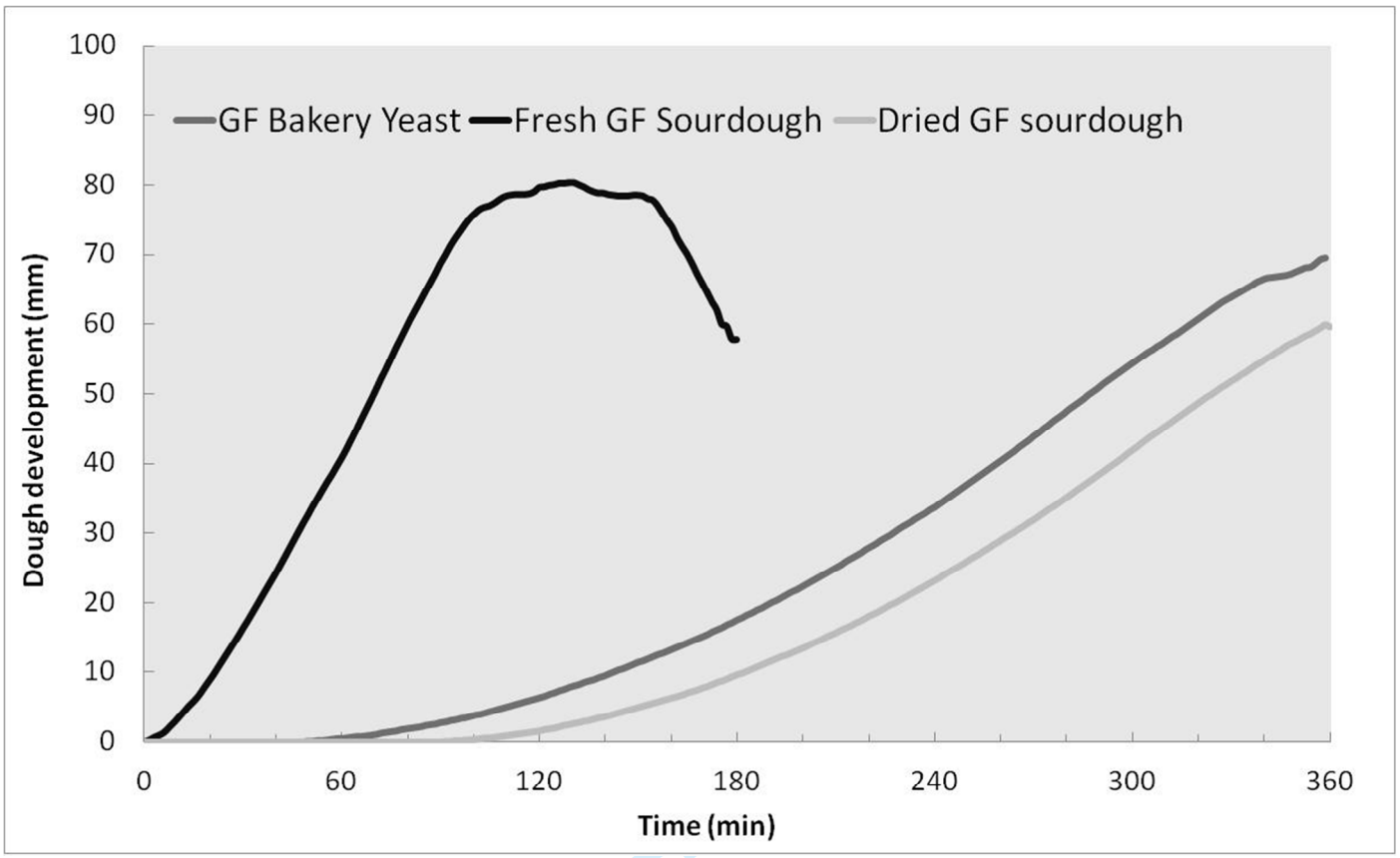
From wheat sourdough to gluten-free sourdough: a non-conventional process for producing gluten-free bread

Journal:	*International Journal of Food Science and Technology*
Manuscript ID:	Draft
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
Complete List of Authors:	Marti, Alessandra; Università degli Studi di Milano, DeFENS, Department of Food, Environmental and Nutritional Sciences Bottega, Gabriella; Università degli Studi di Milano, DeFENS, Department of Food, Environmental and Nutritional Sciences Franzetti, Laura; Università degli Studi di Milano, DeFENS, Department of Food, Environmental and Nutritional Sciences Morandin, Francesca; Università degli Studi di Milano, DeFENS, Department of Food, Environmental and Nutritional Sciences Quaglia, Lucio; Molino Quaglia, Pagani, M. Ambrogina; Università degli Studi di Milano, DeFENS, Department of Food, Environmental and Nutritional Sciences
Keywords:	Cereals and Grains, Pasta, Baking



graphical abstract

From wheat sourdough to gluten-free sourdough: a non-conventional process for producing gluten-free bread

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Abstract

Gluten-free (GF) sourdough was prepared from wheat sourdough, and analyzed both in fresh (GFS) and dried forms (DGFS). The gluten content in each GF sourdough sample was less than 20 mg/kg. The dough leavening capacity and the properties of the bread samples were investigated and compared to those of bread prepared using bakery yeast (*Saccharomyces cerevisiae*). In GFS, Lactic Acid Bacteria (LAB) and yeasts were found in amounts corresponding to 10^8 and 10^7 CFU/g, respectively; whereas, both LAB and yeasts were detected in lower amounts (about 10^6 CFU/g) in DGFS. When used in bread-making, both GFS types produced significant dough acidification and exhibited good dough development during proofing, resulting in loaves with specific volume values between 3.00 and 4.12 ml/g, values similar to those obtained for reference bread (3.05÷4.15 ml/g). The use of GFS was effective in lowering the bread staling rate during storage for up to 7 days.

Keywords: gluten-free sourdough, gluten-free bread, dough leavening, rheofermentometer, bread staling.

Introduction

Celiac disease is one of the most common lifelong disorders affecting approximately 1% of the world's population (Catassi and Fasano, 2008). Since the removal of gluten from the diet results in an improvement in the clinical symptoms of celiacs, the consumption of wheat, barley and rye-based products should be avoided in a gluten-free (GF) diet.

The growing interest in GF foods has stimulated the creation of products that meet the needs of celiacs and their families, as well as those of a large number of consumers who have decided to exclude gluten from their diet for reasons of *health benefits*. Despite a wide variety of breads made from rice, corn, and other GF flours currently available on the market, most of these products are poor in quality (low specific volume, high crumb hardness and crumbling), particularly when compared with their wheat counterparts (Hager *et al*., 2012). Indeed, gluten is responsible for the unique viscoelastic properties (extensibility, resistance to deformation, mixing tolerance and gas-holding capacity) of wheat dough. Consequently bread represents the most challenging GF products to formulate and produce, as gluten is its *architectural key*.

In the past decades, several approaches have been investigated - and recently reviewed by Houben *et al*. (2012) - for the development of GF baked products, such as the use of: *i*) different GF flours (rice, sorghum, oat, buckwheat, amaranth, quinoa, teff, corn); *ii*) ingredients/additives (starches, dairy products, egg proteins, dietary fibre, gum and hydrocolloids); *iii*) alternative technologies such as physical, enzymatic or microbic pre-processing. As regards the last approach, it has already been proved that the use of sourdough in GF bread improves bread texture, extends shelf life and is more flavorful (Zannini *et al.*, 2012). Although the positive contribution of sourdough could produce high quality GF bread, only a few attempts have been made to produce GF sourdoughs and characterize their functional properties. To the best of our knowledge, all these studies investigated the development of sourdoughs from GF cereals or pseudocereals either using selected starter

cultures (Sanni *et al.*, 1998; Schober *et al.*, 2007; Edema and Sanni, 2008) or by spontaneous fermentation of strains isolated from the GF flours (Moore *et al.*, 2007, 2008; Di Cagno *et al.*, 2008; Vogelmann *et al.*, 2009). Exogenous starter cultures are less suitable for the fermentation of GF materials, since the adaptability of the starter strains to the GF sourdoughs is greatly influenced not only by technological parameters but also by the flour and the interactions between starter microorganisms and natural microbiota (Vogelmann *et al.*, 2009; Moroni *et al.*, 2010a, b). The second approach - fermentation of strains isolated from GF flours – involves specific skills, difficult to be transferred to an industrial scale. Finally, Di Cagno *et al.*, (2002) showed that selected LAB, possessing proteolytic activities, could efficiently hydrolyze the toxic peptides of gliadin in wheat sourdough. Breads produced with this sourdough approach exhibited acceptable quality and resulted in no alterations to baseline values of celiac individuals when consumed (Di Cagno *et al.*, 2004). Even if prolonged sourdough fermentation of wheat using specific LAB represents an interesting alternative technology for baking good-quality breads that can be consumed by celiacs, food industries will have to face the obstacle of winning the acceptance of consumers for GF products containing detoxified wheat (Moroni *et al.*, 2009).

Thus, considering the issues related to the current approaches used for sourdough preparation, the aim of this study was: *i*) to propose a method for producing GF sourdough directly from a conventional and strengthened wheat sourdough, removing gluten and, at the same time, maintaining the LAB and yeasts originally present in the wheat sourdough; *ii*) to verify whether the use of the GF sourdough - either fresh or after drying - could improve the characteristics of GF bread prepared without further addition of *Saccharomyces cerevisiae*. To better understand the effects and the possible benefits of baking with sourdough, the characteristics of GF bread samples were compared with those of a reference bread made with commercial bakery yeast and the same GF flour mixture.

Materials and Methods

GF flours

Two commercial GF blends, labeled Mix A and Mix B, differing in protein source, provided by Molino Quaglia S.p.A. (Vighizzolo D'Este, Italy), were used for preparing GF bread. As reported on their labels, Mix A was composed of rice flour, wheat starch, powder milk, sugar, guar flour, and psyllium; Mix B contained rice flour, wheat starch, buckwheat flour (37%), powder milk, sugar, guar flour, and psyllium.

The composite traits of GF flours are shown in Table 1. Starch and soluble sugars, proteins, and total dietary fibre were determined according to the approved methods AACC 44-15, 76-13, 46-12, and 32-05.01 respectively (AACC, 2001).

GF Sourdough preparation

Fresh Gluten free sourdough (GFS) was prepared using a GF *inoculum* obtained directly from a conventional wheat sourdough (WS). WS was maintained in spring water for 24 hours at room temperature; after that it was removed and the water was added to mix A or mix B (water:flour ratio = 60 :100). After a first dough fermentation step (24 hours at 20°C), fresh spring water and GF flour were added to the fermented dough, and the resultant dough was fermented for 24 hours at 20°C. The refreshment step was daily repeated at least 5 times, obtaining GF *inoculum* (GFI) and continued until use. GFI was used as such or after drying (30°C for 36h), resulting in a dried GF *inoculum* (DGFI).

GF bread-making

From fresh Gluten-Free Sourdough (GFS)

GFI was mixed with GF flour (mix A or mix B; GFI: GF flour ratio of 100 :100) and to water (GFI:water ratio of 100 : 70) (Fig. 1a). A first fermentation stage was carried out for 3 hours in an proofing chamber at 30 °C and 85% RH. The refreshment step was carried out twice, obtaining GFI_2 which was added to flour (GFI_2:GF flour ratio of 100 :500) and water (GFI_2:water ratio of 100:400), and the fermentation stage was carried out for 15 hours at room temperature (GF_3). After that, the dough was added to GF mix A or GF mix B (GFI_3:GF flour ratio of 100: 40) and water (GFI_3:water ratio of 100 :50), and mixed in an automatic spiral mixer (Bomann, Clatronic s.r.l., Italy), for 9 min at low speed and for 3 min at high speed. Immediately after mixing, the dough was left to rest for 20 min at room temperature. The final sourdough was labeled as Gluten-Free Sourdough (GFS). The dough was divided into portions of 80 g, moulded into cylinders, put into baking pans (8×4×3.5 cm) and left to rest in a proofing chamber at 30 °C and 85% RH. The proofing time lasted 4 hours in the case of GFS and DGFS; 45 min for BY. All the samples were baked for 1 hour at 185 °C in an oven (Self Cooking Center®, Rational International AG), with vapour injection in the first 20 min of baking (Fig. 1a). Two hours after /removal from the oven the samples were packaged in a perforated OPP film and stored at controlled conditions (20 °C, 60% RH) for seven days.

From Dried Gluten-Free Sourdough (DGFS)

The dried gluten-free *inoculum* (DGFI) was pre-fermented in water (DGFI:water ratio 100:30) for 19 hours in a proofing chamber at 30 °C and 85% RH (Fig. 1b). The resultant dough was added to GF flour (mix A or mix B; $DGFI_2$:GF flour ratio of 200:100) and water ($DGFI_2$:water ratio of 100:50) and the fermentation stage was carried out for 12 hours at room temperature. The dough was added to GF flour ($DGFI_3$:GF flour ratio of 100:400) and water

(DGFI$_3$:water ratio of 100:100) and mixed in an automatic spiral mixer (Bomann, Clatronic s.r.l., Italy), for 9 min at low speed and for 3 min at high speed. Immediately after mixing, the dough was left to rest for 20 min at room temperature. The final dough was labeled as Dried Gluten-Free Sourdough (DGFS). It was transformed into GF bread by adopting the same conditions described for GFS and showed in Fig. 1b.

From Bakery yeast (BY)

Mix A or mix B were mixed with bakery yeast (3g/100g flour) previously dissolved in water. The GF blends/water ratio used for bread-making was 100:100 (Fig. 1c). As for GFS and DGFS, BY was left to rest for 20 min at room temperature after mixing. It was transformed into GF bread by adopting the same conditions described for GFS bread and showed in Fig. 1c.

Sourdough characterization

Chemical characterization

Total titratable acidity (TTA) was determined on 10 g of sample homogenized with 90 ml of distilled water and expressed as the amount (ml) of 0.1 M NaOH to get pH of 8.5. The pH value was determined by a Crison GPL22 pH-meter (Crison Instruments, Alella, Barcelona, Spain).

Microbial characterization

Ten grams of dough sample was aseptically weighed and suspended in a sterile bag, mixed with 90 mL of sterile 0.85% trypton salt solution and homogenized with a Stomacher Calworth 400 Circulator (PBI International, Milan, Italy) at 230 rpm for 1min. Tenfold progressive dilutions were prepared and the following microbiological determinations were performed: *i)* Total Bacterial Count (TBC) by pour plates on Plate Count Agar (PCA) (VWR GmbH, Darmstadt, Germany), incubation at 30 °C for 48 h (ISO, 2003); *ii)* Total Lactic Acid Bacteria (LAB) by pour plates on de Man Rogosa Sharpe agar MRS (Merck, Darmstadt, Germany) incubation under anaerobic conditions (gas pack) at 30 °C for 48 h (De Man *et al*., 1960); *iii)* yeasts by spread technique on Yeast Glucose Chloramphenicole (YGC) incubation at 30 °C for 48 h (ISO, 1992). All microbiological analyses were carried out in duplicate, and the results were expressed as the mean CFU per gram.

Gluten content

The gliadin content measurement was carried out by using a monoclonal R5-antibody-based sandwich enzyme-linked immunosorbent assay (ELISA), RIDASCREEN® Gliadin test kit (R-Biopharm AG, Darmstadt, Germany). Assays were performed according to the standard procedures suggested by the kit supplier. An aliquot of 0.25 g of dough was suspended in 2.5 mL cocktail solution (6 M guanidine chloride and 100 mM 2-mercaptoethanol) and shaken for 40 min at 50 °C. The suspension was then centrifuged at 2500 × *g* for 10 min. The clear supernatant was directly used for immunoassay after 500-fold dilution with a proper dilution buffer. Gliadin contents in all samples were detected in two duplicate and independent measurements, using two different lots of the kit. The gluten content was expressed as the duplicate of the detected gliadin value.

Dough characterization : rheofermentographic test

The dough development and the gas volume produced by GFS, DGFS and BY activities were assessed with a rheofermentometer (Chopin, Tripette & Renaud, Villeneuve La Garenne Cedex, France). Each dough was prepared as described in the "Bread preparation" section. The rheofermentographic test was performed on 315 g portion of the dough and carried out at 30 °C for 3 h when BY was used, and for 6 h when either GFS or DGFS was used. Maximum dough height (Hm; mm), final height of dough (h; mm), maximum height of gaseous production (H'm; mm), time when the porosity of the dough developed (Tx; min), total CO_2 production (CO_2-TOT; ml), CO_2 retained (CO_2-RET; mL), CO_2 released (CO_2-REL; mL), and CO_2 retention coefficient (RC, %) were determined.

Bread characterization

Weight, volume, and specific volume

The apparent volume (n=5) was determined by the rapeseed displacement method, two hours after baking. The weight of the bread (n=5) was recorded and the specific volume was determined through the volume/mass ratio and expressed in mL/g.

Crumb moisture and water activity

The moisture of the crumb core was determined in triplicate using a single-stage drying process for 16 h at 105 °C. The crumb core water activity (aw) was measured in triplicate by an electronic hygrometer (Aqua Lab, CX-2 – Decagon Devices, Pullman, WA).

Crumb texture

Crumb texture characteristics were assessed using a dynamometer (Z005, Zwick Roell, Ulm, Germany), equipped with a 100 N and 5 kN load cell. The three central slices (1.5 mm thickness) of each loaf were compressed to 40% of their height to evaluate hardness, using a cylindrical aluminum probe of 30 mm diameter and a test speed of 2 mm/s. Crumb hardness was measured (n =6) after 0, 1, 2 and 7 storage days and expressed as the load (N) at 30% strain. The rate of staling was calculated as follows: (Firmness after *n* days - initial Firmness)/ initial Firmness; where *n* represents the storage days.

Statistical analysis

The data were processed by Statgraphic Plus for Windows v. 5.1. (StatPoint Inc., Warrenton, VA, USA). A one-way analysis of variance (Anova) was performed using the Least Significant Differences (LSD) test to compare the sample means; differences were considered significant at $P < 0.05$.

Results and Discussion

Sourdough characterization

According to European legislation (EC, 2009) "Foodstuffs may bear the term *gluten-free* if the gluten content does not exceed 20 mg/kg in the food as sold to the final consumer". The process proposed here for preparing a gluten-free *inoculum* (GFI) from wheat sourdough (WS) was effective not only in having a final value for gluten content lower than the legal maximum amount allowed for GF products, but also in maintaining low pH and high acidity

values in the dough in both GF sourdough types (Table 2). In fact, in GFI the LAB and yeast amount was about 10^8 UFC/g and 10^7 CFU/g, respectively, values very close with those measured in WS (Table 2). The drying of GFI allowed the removal of more than 80% of the water such as to guarantee the shelf-life of the product. As expected, drying of sourdough, even if carried out at low temperatures (30 °C for 36 h) caused a lowering in microbial count (Table 2) and, consequently, a lowering of its fermenting capacity. For this reason, when dried starters are used for the sourdough process, the addition of *S. cerevisiae* is more and more frequent in bread-making in order to promote dough development in an acceptable time scale (Corsetti, 2013).

Dough leavening properties

The Rheofermentometer test provides information regarding the gas production and gas holding capacity of dough, useful for predicting the fermentative properties of dough. The rheofermentographic charts and indices of the GF dough samples are reported in Fig. 2 and Table 3, respectively. In both GF mixtures, the presence of hydrocolloids (psyllium and guar flour) assured the formation of a matrix with appropriate consistency for this type of dough according to the farinographic test (150-175 BU). Indeed, a farinographic consistency equal to 200 BU ± 20 was evidenced as the adequate condition to properly form a GF dough able to sustain further transformations, particularly during leavening (Mariotti *et al.*, 2009). The increase in viscosity of the liquid phase, prevented starch and yeast sedimentation and bubble coalescence during fermentation (Mariotti *et al.*, 2013). Nevertheless, the leavening trend differed according to the leavening agent and the GF recipe. As expected, the gaseous production and the amount of CO_2 produced during the leavening phase were higher in BY compared to those prepared with GFS or DGFS (Table 3). In particular, the development of BY dough (Tx, i.e. the moment in which the structure is no longer able to retain the CO_2)

reached the maximum in 120 and 76 minutes, according to the type of GF flour used - A and B, respectively (Fig. 2). Then, this index remained constant and subsequently tended to decrease, following a physiological structural collapse of the dough, responsible for the release of carbon dioxide into the environment.

The development associated with the use of gluten-free sourdough, both GFS and DGFS, markedly differed from that observed in the control (Fig. 2). In particular, the increase in height of the dough containing GFS or DGFS did not show signs of structural failure; on the contrary, these samples were prone to a continuous upward trend even after six hours of fermentation. To summarize: the leavening trend in both sourdough systems was not only similar but also the same height was reached as in BY as long as an extension of proofing time was provided. In fact, at the end of the leavening (360 min), the GFS and DGFS dough exhibited higher (compared to BY) CO_2 retention coefficients, indicating that significant dough expansion is ensured by slow and gradual CO_2 formation. As is well-known, in gluten-based products, gas retention is strongly influenced by the viscoelastic properties of gluten proteins (Cauvain, 2012). In the GF formulations considered in this study, the presence of proteins from milk and/or buckwheat, as well as of hydrocolloids, positively affected CO_2 retention ability. The absence of the Tx index in most of the GF dough samples - even after six hours of fermentation – was the result of a fairly compact mass because the absence of gluten which imparts viscoelastic properties to the dough. Hydrocolloids, in fact, provide proper consistency and compactness to withstand physical stresses but these additives lack the viscoelasticity typical of the gluten network (BeMiller, 2009). Our results agree with those reported in other studies: the time of appearance of the porosity in mixtures containing sourdough is superior to non-acid doughs (Dal Bello *et al.*, 2007).

Regarding the recipe, the presence of buckwheat flour negatively influenced dough leavening: 40% lower height in mix B compared to mix A. This result is likely due to a weakening of the protein network in the presence of buckwheat flour (Torbica *et al.*, 2010).

On the contrary, Mariotti *et al.* (2013) showed improvements in dough development with the incorporation of buckwheat likely because of an increase in dough viscosity, as a consequence of its high dietary fiber content.

Bread characteristics

The properties of GF breads obtained from the different leavening agents (BY, GFS, and DGFS) are reported in Table 4. In agreement with the rheofermentometer data, the best bread-making performances were obtained with mix A, which exhibited higher height and specific volume indices than mix B. The presence of buckwheat flour, indeed, could enhance the nutritional value of the bread but at the expense of poor bread volume, as referred by Moore *et al*. (2004) and Moore *et al.* (2009). Despite that, the bread characteristics obtained for mix B products were in the range of acceptable loaf volume, and comparable to those reported in the literature (Mezaize *et al.*, 2009; Mariotti *et al.*, 2013).

Regardless of the mixture composition, bread samples prepared using BY or GFS did not show significant differences ($p>0.05$) in crumb moisture and water activity (Table 4). The bread development indices were in agreement with literature data (Moore et al., 2007; Schober *et al.*, 2007; Mariotti *et al.*, 2013) and suggested that the sole use of GFS resulted in bread with a specific volume comparable to that obtained using *S. cerevisiae* (Table 4). The improvement of GF bread by sourdough was less noticeable when DGFS was used, thus suggesting the need to combine the type of sourdough with bakery yeast.

As regards to crumb firmness, samples from mix B were characterized by a higher initial consistency than samples from mix A (Table 4), probably due to the presence of buckwheat. However, the use of this raw material induced a decrease in crumb softness, due to its richness in non starch polysaccharides (Biacs *et al.*, 2002). At the same time, the high hydrophilic characteristics of fibre components resulted in a lower staling of the product

over time (Fig. 3). The effect of using GFS on crumb texture during storage was more evident when mix A was used. Although bread from sourdough fermentation exhibited higher initial firmness compared to bread with *S. cerevisiae*, the use of either GFS or DGFS resulted in GF breads characterized by longer shelf-life, in agreement with previous studies (Corsetti *et al.*, 1998; Corsetti *et al.*, 2000; Schober *et al.*, 2007).

Conclusions

The present study shows that it is possible to obtain GF sourdough from wheat sourdough, suitable to produce bread without adding *S. cerevisiae* or selected cultures of LAB. It has been proved that the use of the GF sourdough dried at low temperatures contains alive and vital microbial strains (LAB and yeasts). The LAB and yeasts present in GF sourdough assured an appropriate development of the dough during proofing, resulting in bread with a high specific volume, similar to that observed when bread was prepared with BY only. Finally, the use of GF sourdough, either as such or after partial dehydration, resulted in bread characterized by better shelf-life over time, especially for the formulation composed mainly of starchy material.

Acknowledgements

The Authors would like to thank Prof. Stefania Iametti and her team for gluten determination. The Authors declare that there is no conflict of interest.

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Figure legends

Fig. 1. Dough and bread preparation using gluten-free sourdough *inoculum* (a), dried gluten-free sourdough *inoculum* (b), and bakery yeast (c).

Fig. 2. Rheofermentographic curves of dough development of GF mix A (a) or mix B (b).

Fig 3. Rate of staling of gluten-free doughs prepared from mix A (a) or mix B (b). The rate of staling was calculated as [(Firmness after *n* days - initial Firmness)/ initial Firmness], where *n* represents the storage days. The detail in panel (b) represents an enlargement of the picture.

Table 1. Chemical composition of GF blends (g/100 g d.b.)

	Mix A	Mix B
Starch and soluble sugars	82.4	79.0
Protein	5.8	7.0
Fibre	3.8	6.0

d.b. = dry basis

458 Table 2. Gluten-free sourdough characterization.

	Wheat Sourdough (WS)	Gluten-free *Inoculum* (GFI)	Dried gluten-free *Inoculum* (DGFI)
Moisture (g/100g)	53.0 ± 0.06	49.1 ± 0.41	9.0 ± 0.05
pH	3.81 ± 0.02	4.11 ± 0.04	4.37 ± 0.02
Total titratable acidity (ml NaOH 0.1M / 10g)	8.78 ± 0.32	7.73 ± 0.57	7.20 ± 0.52
Total Bacteria Count (CFU/g)	$8*10^8$	$9*10^8$	$8*10^7$
LAB (CFU/g)	$7*10^8$	$8*10^8$	$9*10^6$
Yeast (CFU/g)	$8*10^7$	$9*10^7$	$9*10^6$
Gluten (mg/kg)	>300	< 20	< 20

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461 Table 3. Dough rheofermentographic indices.

	Leavening agent	Hm (mm)	h (mm)	H'm (mm)	Tx (min)	CO_2-TOT (ml)	CO_2-RET (ml)	CO_2-REL (ml)	RC (%)
Mix A	BY	80.3	57.8	89.6	91	1688	1547	141	91.6
	GFS	69.6	69.6	31.9	-	1018	1011	7	99.3
	DGFS	59.9	59.6	47.0	-	618	612	6	99.0
Mix B	BY	48.3	35	85.9	76	1812	1509	303	83.3
	GFS	40.1	28.7	39.7	256	1237	1188	49	96.0
	DGFS	44.7	44.7	2.5	-	1051	1040	10	99.0

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463 Hm = maximum dough height; h = final height of dough; H'm = maximum height of gaseous

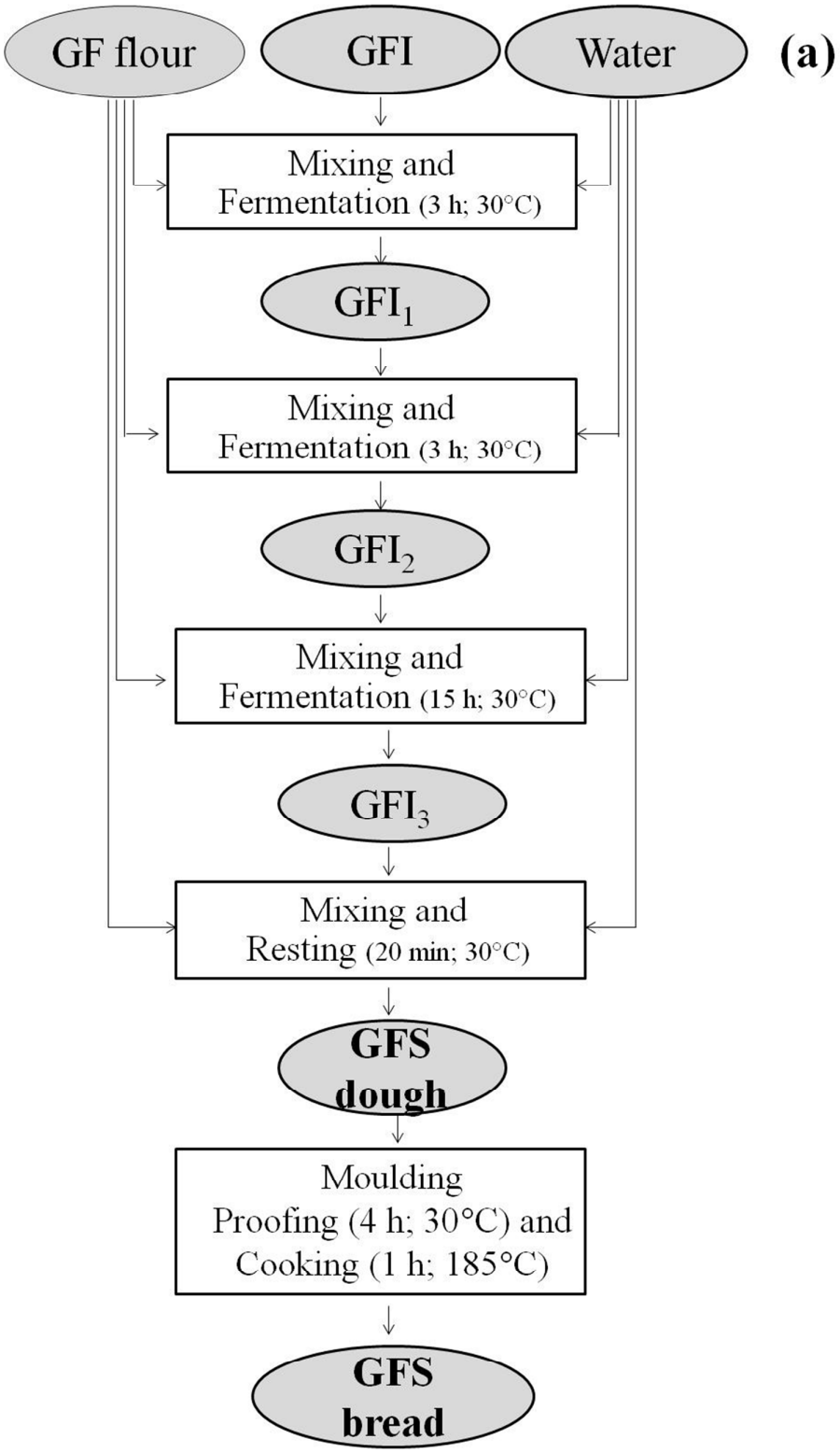
464 production; CO_2-TOT = total CO_2 production; CO_2-RET = CO_2 retained; CO_2-REL = CO_2

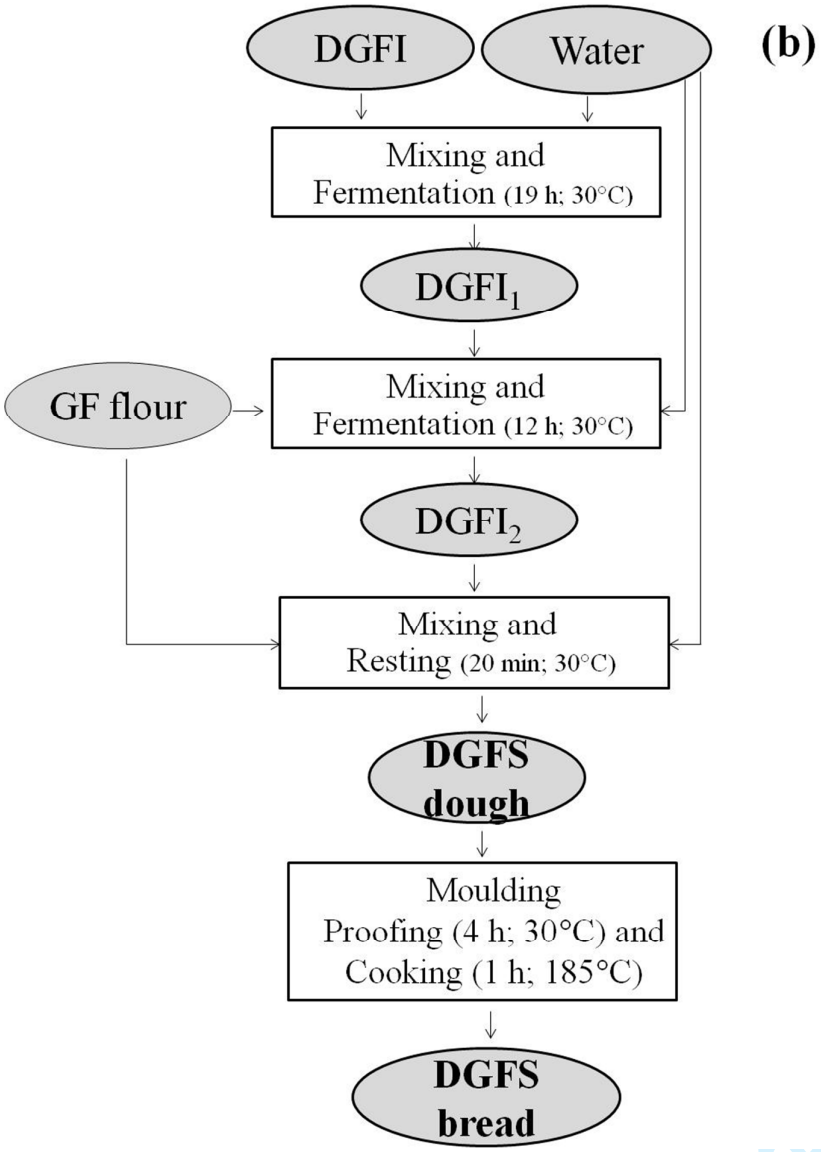
465 released; RC = CO_2 retention coefficient; Tx = porosity time

Table 4. Bread characteristics.

	Leavening agent	Weight (g)	Height (cm)	Specific volume (cm^3/g)	Crumb moisture (g/100g)	Crumb a_w	Firmness (N)
Mix A	BY	55.9±1.3b	6.53±0.12e	4.15±0.22c	53.9±0.75a	0.98±0.001a	2.22±0.24a
	GFS	47.3±1.7a	5.57±0.12d	4.12±0.14c	54.1±2.7a	0.98±0.005a	4.86±1.51ab
	DGFS	54.5±0.6b	5.23±0.06c	2.97±0.07a	54.6±0.25a	0.99±0.002a	10.58±0.53d
Mix B	BY	54.8±0.9b	4.97±0.12b	3.22±0.10b	53.8±0.73a	0.99±0.006a	6.63±1.26bc
	GFS	55.9±0.2b	4.97±0.06b	3.10±0.08b	53.8±0.53a	0.98±0.005a	7.33±1.15c
	DGFS	58.1±0.7c	4.70±0.10a	2.95±0.06a	54.9±0.16a	0.98±0.004a	6.86±1.20c

Means and standard deviations followed by different letters in a column are significantly different (LSD; $p<0.05$)


GF flour
GFI
Water
(a)
Mixing and Fermentation (3 h; 30°C)
GFI1
Mixing and Fermentation (3 h; 30°C)
GFI2
Mixing and Fermentation (15 h; 30°C)
GFI3
Mixing and Resting (20 min; 30°C)
GFS dough
Moulding
Proofing (4 h; 30°C) and
Cooking (1 h; 185°C)
GFS bread


(b)
DGFI
Water
Mixing and Fermentation (19 h; 30°C)
DGFI1
GF flour
Mixing and Fermentation (12 h; 30°C)
DGFI2
Mixing and Resting (20 min; 30°C)
DGFS dough
Moulding
Proofing (4 h; 30°C) and Cooking (1 h; 185°C)
DGFS bread

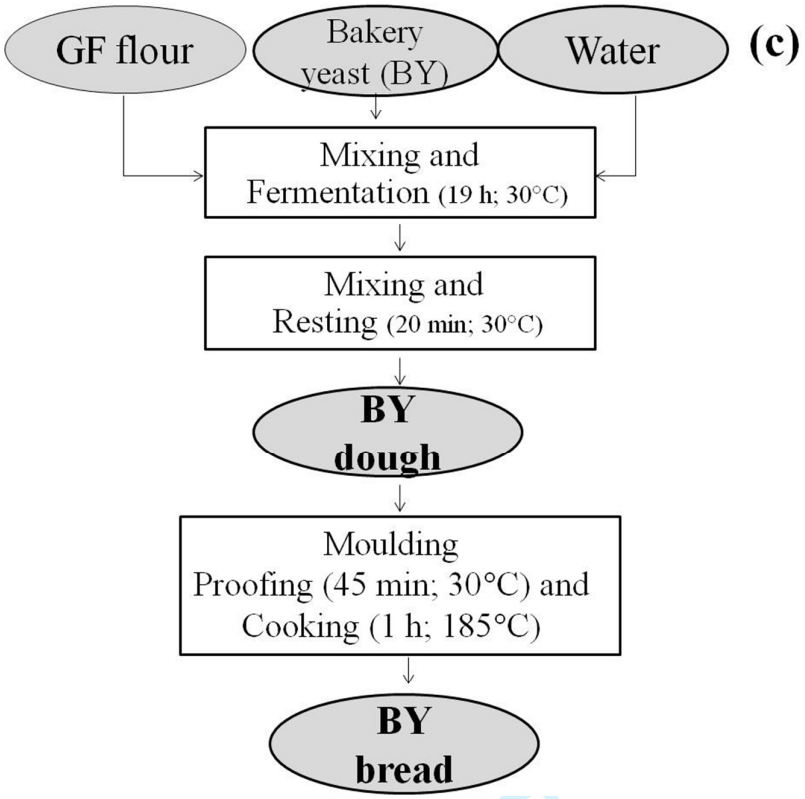


Fig. 1.

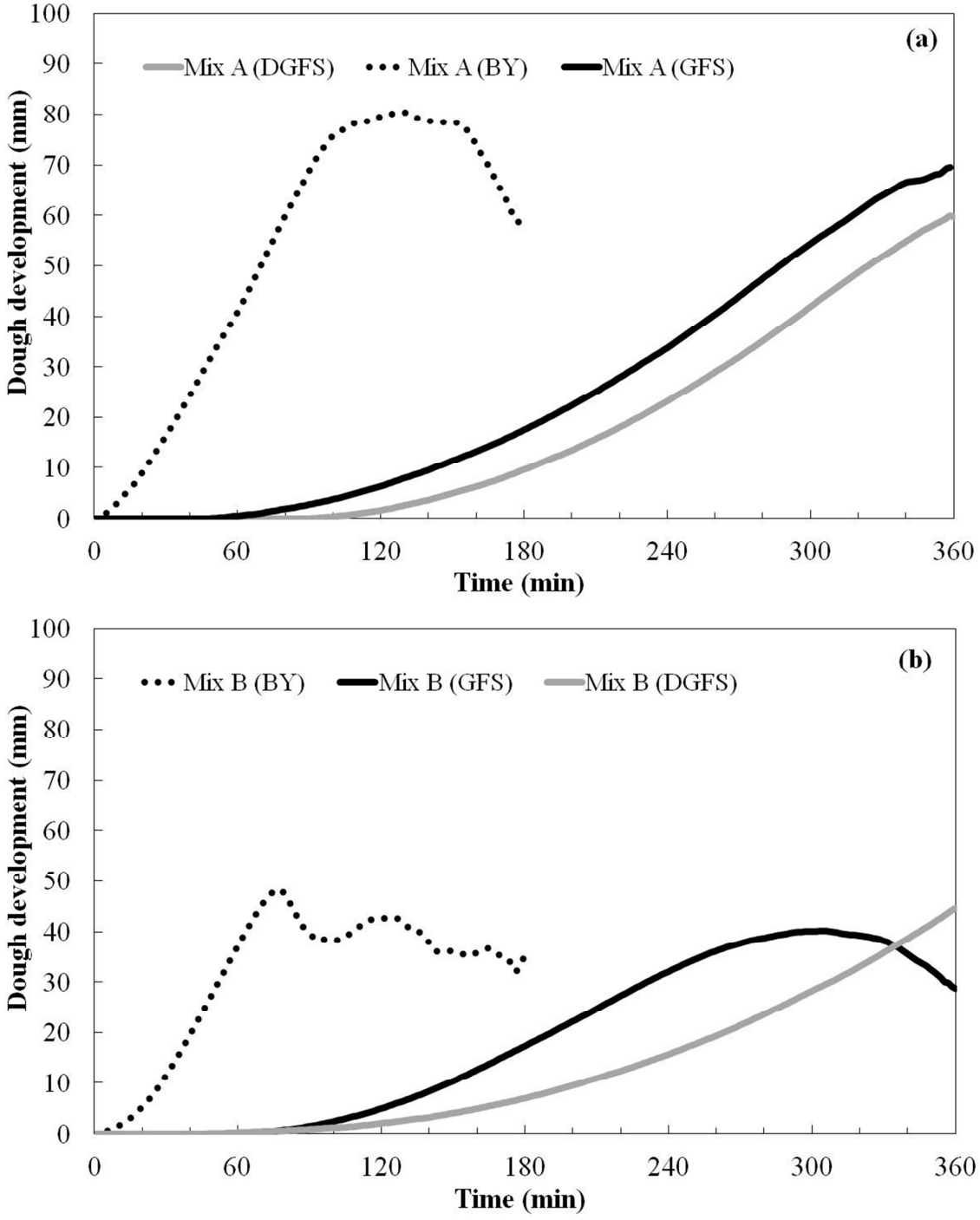

(a)
Mix A (DGFS)
Mix A (BY)
Mix A (GFS)
Dough development (mm)
Time (min)
0
10
20
30
40
50
60
70
80
90
100
0
60
120
180
240
300
360
(b)
Mix B (BY)
Mix B (GFS)
Mix B (DGFS)
Dough development (mm)
Time (min)

Fig. 2

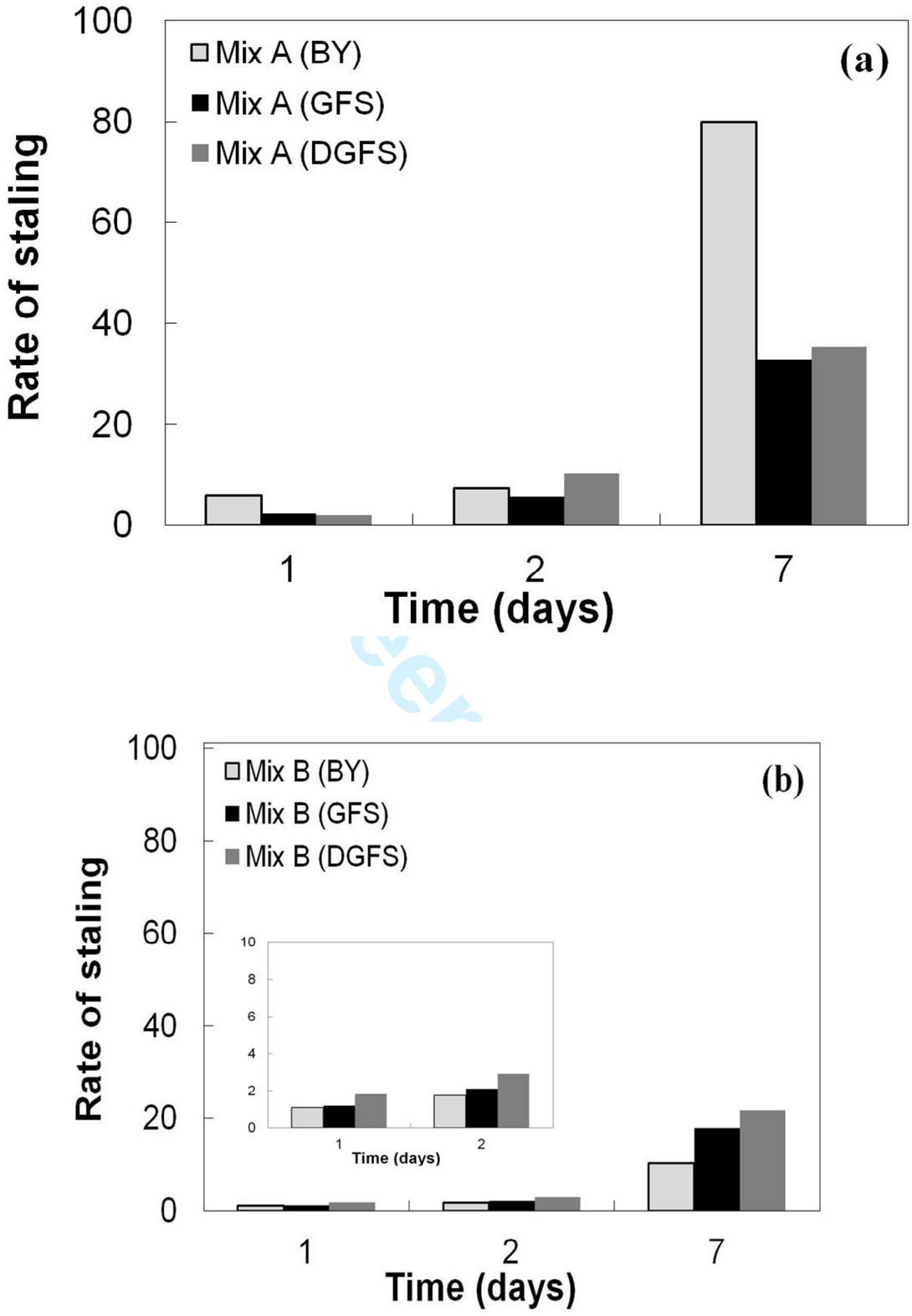

100
80
60
40
20
0
Rate of staling
Mix A (BY)
Mix A (GFS)
Mix A (DGFS)
(a)
1
2
7
Time (days)
100
80
60
40
20
0
Rate of staling
Mix B (BY)
Mix B (GFS)
Mix B (DGFS)
(b)
10
8
6
4
2
0
1
2
Time (days)
1
2
7
Time (days)

Fig. 3.