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Original paper

Evolution of biochemical and rheological characteristics and breadmaking quality during a multistage wheat sour dough process *

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Entwicklung biochemischer und rheologischer Eigenschaften und des Backverhaltens während einer mehrstufigen Weizensauerteigführung

Zusammenfassung. Es wurde die Entwicklung biochemischer Daten (Essigsäure- und Milchsäuregehalt, Gärungsquotient und Veränderungen an Mono-, Di- und Trisacchariden, rheologischer und Gäreigenschaften (Extensograph, Maturograph und Ofentrieb) und der Brotqualität (Volumen, Dichte, Textur und Säuregrad) während einer mehrstufigen Weizensauerteigführung untersucht. Die Essigsäuregehalte von Sauerteigen, Brotteigen und Broten stiegen mit der Zahl der Führungsstufen an. Beim Milchsäuregehalt ergaben sich ähnliche Tendenzen, jedoch beim Brotteig und Brot wurde das Maximum erst in der dritten Stufe erreicht. Veränderungen des Zuckergehaltes stimmten mit der Gäraktivität und der Mikrofloraentwicklung in den verschiedenen Stufen überein. Es ergab sich eine Verbesserung in den rheologischen und Gäreigenschaften von Brotteigen sowie im Ofentrieb, wenn die Zahl von Sauerteigstufen anstieg. Es wurde auch ein positiver Einfluß auf die Broteigenschaften (Volumen, Dichte und Säuregrad) beobachtet. Die letzte Stufe der Sauerteigführung ergab das beste Endprodukt mit deutlichen Unterschieden, wenn mit einer geringeren Zahl von Stufen hergestellt.

Summary. The evolution of some biochemical (acetic and lactic acid contents, fermentation quotient and changes in mono-, di-, and trisaccharides), rheological and fermentative characteristics (extensigram, maturogram and impulsogram) and bread quality (volume, density, texture, and degree of acidification) during a multistage wheat sour dough process have been investigated. Acetic acid contents of sour doughs (SD), bread doughs (BD) and breads (B) increased with the increase in number of processing stages. Lactic acid followed similar trends in SD, but in BD and B it reached maximum levels during the third stage. SD contained higher amounts of acids than BD and B. Dynamics of sugars followed patterns according to the fermentative activity and microflora evolution in the different steps. An improvement as the number of SD stages increased was observed in rheological and fermentative properties of BD, such as in oven spring. A positive effect on B characteristics (volume, density, texture, and acidity) was also observed. The last stage of the SD process led to the best final product, with noticable differences in relation to those occurring with a smaller number of stages.

Introduction

The use of wheat flour sour doughs elaborated by multistage processes is a common procedure in several countries [1–7] and, if properly conducted, leads to high-quality breads [4]. It has been well established that the "mother" dough improves the rheological and fermentative characteristics of doughs [1]. However, the main contribution of sour "mother" doughs is their active microflora, together with a series of active elements originating from their own microbial metabolism and from the enzymatic activities of flour. These constituents contribute to progressively modify the environment determining as much dough properties as microflora activity.

The combination of yeast and lactic acid bacteria in doughs fermented over a long period of time results in important leavening and souring actions that positively influence the flavour and texture of the bread. Organic acids increase the dough acidity and modify physicochemical characteristics of wheat gluten [8–10]; thus some rheological properties of doughs such as water absorption, mixing tolerance, dough consistency [9, 11] and resistance to extension [8] increase, whereas others such as mixing time, stability [11] and extensibility [10] decrease.

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By lowering the pH, organic acids influence redox processes and, in general, the numerous biochemical reactions occurring during fermentation [12]. Additionally, the development of microbial groups is affected; spoiling microorganisms are inhibited [13, 14] and a selection of species is promoted.

Multistage sour dough processes are complex and time consuming. Fermentation periods between "refreshing" steps last between 18 and 24 h; over this period, microflora undergo continuous environmental changes (pH, redox potential, nutrient disponibility, catabolite formation) that doubtless have a direct effect on their activity and development, and indirectly upon the biochemical and functional characteristics of doughs.

In previous papers [15, 16] microorganisms populating the different steps of a multistage sour dough process have been identified, and their evolution determined. Significant variations in microflora were detected mainly during the sour dough steps. Yeast counts did not change during the first sour dough fermentation ($\simeq 2 \times 10^3$ cfu/g), but increased continuously through the next stages up to 4.9×10^6 cfu/g. Lactic acid bacteria counts increased from 2.5×10^3 cfu/g (first stage) to 6.2×10^8 cfu/g (second stage) and remained unchanged until the end of the process. The most prevalent yeast was *Saccharomyces cerevisiae*, followed by *C. boidinii* (third stage). Lactic acid bacteria underwent greater changes. During the first sour dough stage, only homofermentative lactobacilli were isolated, but after fermentation their number decreased and heterofermentative species appeared, thus increasing the population of cocci (homo- and hetero-). This tendency reached a maximum during the third stage, where about 80% of colonies belonged to homofermentative cocci. Lactobacilli were not found. Finally, in the last sour dough stage, a balanced population consisting of homo- and heterofermentative lactobacilli ($\simeq 90\%$) was developed. In bread doughs, a similar evolution of lactic acid bacteria species was observed.

This work continues previous paper and studies: (a) the evolution of sugars and organic acids (acetic and lactic acid) in each step; (b) the influence and degree of sour dough fermentation at the different stages on the biochemical, rheological and fermentative characteristics of dough made thereof and on the physicochemical properties of bread; and (c) relationships between microflora composition and evolution and sour and bread dough characteristics.

Material and methods

The multistage process (1–5 steps) used to produce wheat sour doughs (SD) and bread doughs (BD) was carried out following the procedure previously described [4, 15, 17] and has been summarized in Table 1. Each SD fermentation period lasted 24 h at 28° C and 85%–90% relative humidity (RH).

The breadmaking test was performed as follows: doughs underwent a bulk fermentation step to a volume increase of 1.8 (2–3 h) in a fermentation cabinet set at 28° C and 85% RH. Sixty grams of scaled pieces was rounded, left to stand for 12–15 min, hand-shaped and proofed to an optimal volume increase (1.5–3 h). Baking was carried out at 200° C for 25 min.

Acetic and lactic acids were determined by enzymatic methods as reported by Martínez-Anaya et al. [18]. Sugars (mono-, di-, and trisaccharides) were determined as methyl-silyl derivates as described previously [18].

The spread test was performed according to Hoseney et al. [19], with the modifications described by Barber et al. [20]. Extensogram, maturogram and impulsogram were recorded following the recommendations of Brabender (Brabender, DHG, Duisburg, FRG). Bread characteristics [volume, density, texture (maximum deformation strength; Instron), total titratable acidity, and acetic and lactic acids] were determined by previously established procedures [18, 21, 22].

Results and discussion

1 Evolution of biochemical characteristics

1.1 Organic acids

1.1.1 Acetic acid. The acetic acid level in unfermented SD during the first stage (USD1) was 0.069% (g acid/100 g dough dry weight), a value that steadly increased after each mixing step, up to 0.125% at the fifth stage (USD5) as shown in Fig. 1 a. Amounts of a similar order to that of USD1 were observed in other unfermented doughs made with yeast and/or lactic acid bacteria [18, 21, 22], and they were attributable mainly to flour acidity. Over a period of 24 h in the SD fermentation (FSD) acetic acid increased in variable amounts depending on the SD stage. FSD1 reached the lowest level (0.106%) and FSD5

Table 1. Characteristics of the multiple stages sour dough manufacturing process

	Formulation (%)						
	Sour dough (SD)					Bread dough (BD)	
Stage	1	2	3	4	5		
Wheat flour	100	100	100	100	100	Flour	100
Bran extract	50	–	–	–	–	Dry yeast	0.37
Water	–	43	43	43	43	Water	63
Salt	0.5	0.5	0.5	0.5	0.5	Salt	2
Sucrose	–	–	0.5	–	–	SD	30
Previous sour dough	–	100	100	100	100		

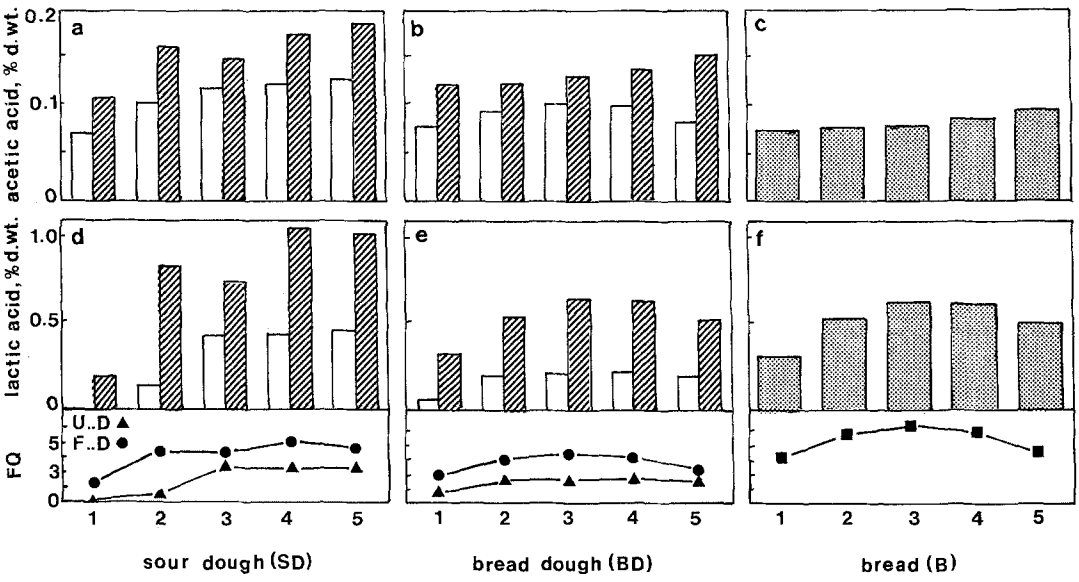

Fig. 1a–f. Acetic and lactic acid contents and fermentation quotient (*FQ*) of sour doughs (*SD*), bread doughs (*BD*) and breads (*B*) on a multistage wheat sour dough process. *White bars:* immediately after mixing (*UD*); *hatched bars:* after fermentation (*FD*); *shaded bars:* bread

the highest (0.184%). However, total increase due to fermentation were greater in FSD2 and FSD5 (=0.060%, Fig. 1 a). These results could be correlated to microbial activity in SD; SD1 gave low yeast counts at this stage and SD3 contained the highest proportion of homofermentative lactic acid bacteria species. During the second mixing stage, a considerable increase in the number of both groups of microorganisms occurred so that the development of heterofermentative species took place. Afterwards, the total viable lactic acid bacteria counts remained unchanged, but an evolution of different species was observed [15, 16]. Values of acetic acid in the same range were found by Spicher et al. [23, 24] in wheat SD made by several multistage SD processes.

The acetic acid content of unfermented bread doughs (UBD) at each stage varied from 0.076% (UBD1) to 0.099% (UBD3) and basically corresponded to the amounts furnished by SD and flour. Proofing of BD (FBD) resulted in new increases in acetic acid to final levels ranging from 0.120% to 0.151% (Fig. 1 b). The total amount of this acid (0.030%–0.050%) produced on fermentation reached a minimum in BD3, where the lactic acid bacteria population consisted mainly of homofermentative cocci.

1.1.2 Lactic acid. Immediately after mixing, USD1 contained very low quantities of lactic acid (0.001%), owing to the small number of lactic acid bacteria present [15, 16]; these amounts increased in the following SD mixing stages,until the third stage ($\simeq$0.4%–0.5%) after which no further significant ($P<0.05$) changes were observed (Fig. 1 d). This behaviour correlated well with the lactic acid bacteria development in SD, which reached their maximum at SD3 and did not vary in the latter stages [15, 16].

The level of lactic acid in FSD increased after fermentation from 0.189% (FSD1) to 1.059% in the last two steps (FSD4, FSD5) as shown in Fig. 1 d. The amounts found were close to those reported by other authors [24]. The lactic acid production during SD fermentation were well correlated to microflora evolution during different stages of SD production [15, 16]. During the first stage (USD1) about 91% of lactic acid corresponded to the L(+) isomer, but a shift to a higher proportion of D(−)-lactic acid (25%) was observed in FSD1 (Table 2). This could be due to the development of *Leuconostoc* species, which were not previously detected [15, 16]. Except for USD2 the various stages produced percentages of the L(+)-lactic acid ranging from 53% to 64%, the highest value corresponding to FSD.

As it has been shown for acetic acid (Fig. 1 b), during BD production (except for BD1), lactic acid levels in

Table 2. Percentage of L(+)- and D(−)-lactic acid isomers of sour dough (SD), bread dough (BD) and bread (B) on a multistage wheat sour dough process

	L(+)-lactic acid		D(−)-lactic acid	
	Unfermented	Fermented	Unfermented	Fermented
SD 1	91	75	9	25
SD 2	75	63	25	37
SD 3	53	59	47	41
SD 4	54	64	46	36
SD 5	55	62	45	38
BD 1	80	83	20	17
BD 2	57	61	43	39
BD 3	56	60	44	40
BD 4	60	62	40	38
BD 5	64	63	36	37
B 1		83		17
B 2		62		38
B 3		60		40
B 4		62		38
B 5		63		37

UBD and FBD were lower (Fig. 1 e) than those obtained in USD and FSD (Fig. 1 d). From stepts 2 to 5, the respective values of these acids underwent slight changes. UBD1 and FBD1 gave the smallest contents (0.056% and 0.317%, respectively), which increased to 0.180%–0.210% for UBD2–5 and to 0.503%–0.631% after fermentation of FBD2–5 (Fig. 1 e). During the first stage, approximately 80% of this acid corresponded to the L(+) isomer, but from the second to the fifth stage, a lower percentage (55%–63%) of this isomer was observed (Table 2).

In rye sour doughs, the fermentation quotient (FQ) (molar ratio lactic acid/acetic acid) plays an essential role with respect to the bread flavour and texture [14, 25]. FSD gave an FQ ranging from 1.18 (FSD1) to 4.08 (FSD4), whereas for FBD they varied from 1.76 (FBD1) to 3.27 (FBD3) (Fig. 1). Although for rye SD optimal FQ values of 1.5–4.0 have been reported [14, 25], the FQ of wheat sour doughs can vary over wider ranges (1.0–9.57) [7, 23, 26] and so far, no recommendations for these doughs have been established. Results of this work point to FQ values for the SD close to 4.0, which leads to the best quality breads (see below); in BD no clear trend could be observed between both parameters.

2 Changes in mono-, di and trisaccharides

2.1 Monosaccharides

The glucose content of USD varied from 0.22% (g sugar/100 g dough dry wt.) to 0.45% and increased after fermentation to 1.82% and 0.60% for the first two stages (Table 3). During the third step the rates of glucose consumption and glucose formation were equal and no changes were detected. Finally, FSD4 and FSD5 showed a decrease in glucose levels of 0.11% and 0.05%, respectively. The evolution in glucose fermentation corresponded to the development of microbial activity. During the first two stages, yeast cells reached counts between 2×10^3 and 1.2×10^4 cfu/g dough wet wt [15, 16]; therefore only limited activity attributable to yeast could be detected. Bacterial activity was also small, as has been shown in organic acid production (Fig. 1). Because of this, the production of glucose as a result of sucrose inversion and enzymic degradation of flour and bran extract oligo- and polysaccharides [15, 16] were greater than its assimilation by the microflora present. During the following steps, the cell counts of microorganisms gradually increased and a noticable tendency for the glucose content to drop was observed, together with a higher gassing power and dough acidity [15, 16].

The fructose content in SD and the evolution was similar to that mentioned for glucose, but it began to disappear latterly during the third stage (Table 3). After each mixing step, the fructose level oscillated between 0.12% (USD1) and 0.59% (USD3) and increased up to 1.33% (FSD2) over a 24 h fermentation period. As expected, the final level of fructose in the last stage of SD fermentation (FSD5) was lower (0.37%), although it exceeded that found for glucose. As it has been previously pointed out,

Table 3. Sugar contents[a] of sour dough (SD) and bread doughs (BD) on a multistage wheat sour dough process

Stage	Glucose		Fructose		Maltose		Sucrose		Maltotriose		Raffinose	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
Sour dough												
SD 1	0.22 ± 0.06[b]	1.82 ± 0.20	0.12 ± 0.01	0.67 ± 0.08	3.95 ± 0.20	4.92 ± 0.39	0.62 ± 0.11	0.57 ± 0.06	ND	0.04 ± 0.01	0.57 ± 0.06	0.17 ± 0.03
SD 2	0.45 ± 0.06	0.60 ± 0.07	0.28 ± 0.01	1.33 ± 0.08	5.33 ± 0.27	8.58 ± 0.34	0.47 ± 0.03	0.04 ± 0.01	ND	0.50 ± 0.23	0.36 ± 0.02	0.04 ± 0.001
SD 3	0.31 ± 0.04	0.30 ± 0.02	0.59 ± 0.06	1.17 ± 0.06	5.57 ± 0.42	7.66 ± 0.54	0.59 ± 0.04	0.09 ± 0.08	0.05 ± 0.007	0.68 ± 0.003	0.27 ± 0.02	0.04 ± 0.003
SD 4	0.28 ± 0.02	0.11 ± 0.009	0.48 ± 0.02	0.63 ± 0.02	6.10 ± 0.33	1.66 ± 0.13	0.21 ± 0.02	0.006 ± 0.007	0.07 ± 0.009	0.39 ± 0.07	0.27 ± 0.03	ND
SD 5	0.40 ± 0.05	0.05 ± 0.02	0.43 ± 0.07	0.37 ± 0.04	2.69 ± 0.19	0.24 ± 0.05	0.06 ± 0.04	0.01 ± 0.01	0.05 ± 0.005	0.09 ± 0.03	0.20 ± 0.01	0.02 ± 0.02
Bread dough												
BD 1	0.72 ± 0.07	0.18 ± 0.07	0.94 ± 0.07	0.59 ± 0.07	4.18 ± 0.39	5.15 ± 0.45	Trace	0.03 ± 0.02	ND	0.05 ± 0.03	0.31 ± 0.05	ND
BD 2	0.82 ± 0.18	0.12 ± 0.01	0.96 ± 0.03	0.56 ± 0.02	4.52 ± 0.12	4.56 ± 0.25	0.02 ± 0.02	0.06 ± 0.05	ND	0.07 ± 0.005	0.16 ± 0.04	ND
BD 3	0.54 ± 0.08	0.06 ± 0.03	0.83 ± 0.08	0.28 ± 0.02	3.73 ± 0.58	3.17 ± 0.33	0.03 ± 0.04	0.04 ± 0.02	ND	0.08 ± 0.02	0.24 ± 0.08	ND
BD 4	0.58 ± 0.01	0.13 ± 0.04	0.81 ± 0.02	0.33 ± 0.05	2.99 ± 0.14	2.66 ± 0.28	0.05 ± 0.06	0.04 ± 0.01	ND	0.01 ± 0.001	0.15 ± 0.01	ND
BD 5	0.60 ± 0.03	0.09 ± 0.006	0.82 ± 0.03	0.21 ± 0.01	3.17 ± 0.07	2.08 ± 0.23	ND[c]	0.05 ± 0.01	ND	0.06 ± 0.01	0.18 + 0.002	ND

[a] g sugar/100 g dough dry wt [b] Standard deviation [c] Not detected

when there are similar levels of glucose and fructose in the dough, the former is metabolized faster [18, 21, 27].

In UBD, glucose and fructose contents oscillated between 0.54%–0.82% and 0.81%–0.96% respectively (Table 3). The highest values corresponded to UBD elaborated with SD from the first two process stages. As previously mentioned, it contained greater quantities of these sugars after SD fermentation (when they were used to formulate BD). In FBD, the amounts of both sugars decreased at every stage; they did not significantly ($P<0.05$) differ for glucose (0.06%–0.18%), but decreased slowly, as the number of steps increased, to 0.21% (FBD5) for fructose. This behaviour is in agreement with the greater fermentative activity existing in BD as the number of process steps increases.

2.2 Disaccharides

The sucrose content of USD1 was 0.62%, and did not vary significantly during SD1 fermentation (Table 3). This agrees with the low yeast counts [15, 16] and therefore with the small invertase activity at this stage. In the subsequent SD steps, the sucrose level after mixing progressively decreased, except in USD3, where 0.5% was included in the formula and only traces were detected in USD5. During fermentation (FSD2–FSD5), as the number of yeasts increased, mainly *Saccharomyces cerevisiae* species developed and sucrose dropped to trace levels. Similar trends were observed with pure strains of *Saccharomyces* genera in the wheat doughs [18, 21].

In BD that contained 0.4% commercial dry yeast [15], only traces of sucrose could be detected in UBD and FBD (Table 3) due to the yeast's invertase activity [12, 28, 29].

Maltose levels in USD increased to 6.10% by the fourth mixing step (Table 3). Accordingly, in comparison to the proportions of the monosaccharides glucose and fructose existing in USD at that time, maltose accumulated in the doughs because its metabolism by yeast, not previously adapted to its fermentation, did not begin until the glucose and fructose levels reached very low values [18, 21, 27, 30]. Thus, enzymatic degradation of wheat flour damaged starch promotes a high concentration of this sugar, as reported by several authors [31, 32]. In a similar way, maltose contents of FSD increased during the first three stages, reaching its maximum at FSD2 (8.58%). From FSD3 to FSD5, a steady decrease in the final concentration of this sugar was observed, in agreement with the dynamics of changes of glucose and fructose. FSD5 contained only 0.24% of maltose, as a result of the active fermentation by the microorganisms developed in SD. After mixing, UBD produced quantities of maltose ranging from 4.52% to 3.17% (Table 3). Extreme values corresponded to the first two and last stages, respectively. In spite of glucose and fructose levels in UBD (Fig. 2 b, d), maltose was metabolized from the second step on, as a result of adaptation of the fermentative microflora to maltose fermentation during the previous SD steps [28, 30].

2.3 Trisaccharides

Although maltotriose appeared at trace levels after mixing, low amounts of this sugar were formed during SD fermentation (Table 3), reaching up to 0.68% for FSD2. It seemed that its production from the amylase action on flour starch exceeded its consumption by the fermentative microflora. In later steps, a trend for this sugar to disappear was observed. When BD were prepared, maltotriose was only detected after proofing and found in trace amounts.

USD1 contained 0.56% raffinose (Table 3), an amount higher than that reported previously in wheat flour [33]. However, after sucrose raffinose is the most abundant sugar in wheat bran [34] and a bran extract was used to make the first SD [15, 16]. Raffinose levels in USD decreased as the multistage process proceeded, becoming stable from USD3 (0.27%) onwards. During SD fermentation raffinose dropped to trace levels in every step.

Noticeable decreases in the trisaccharides were found in spontaneous wheat sour doughs by Saunders et al. [31]. Raffinose was detected in UBD in lower amounts than in USD (0.15–0.30%) and it was hydrolyzed during the fermentation process. These results are in accordance with the development of yeasts observed in SD and therefore with invertase activity and with the addition of a small amount of commercial dry yeast (0.37%) to BD.

3 Evolution of rheological and fermentative properties of BD

3.1 Dough spreading

The width/height ratio of a rounded piece of dough during fermentation gives an estimation of dough stability [19]. Its value increased during fermentation in all BD stages (Fig. 2), following common trends observed by

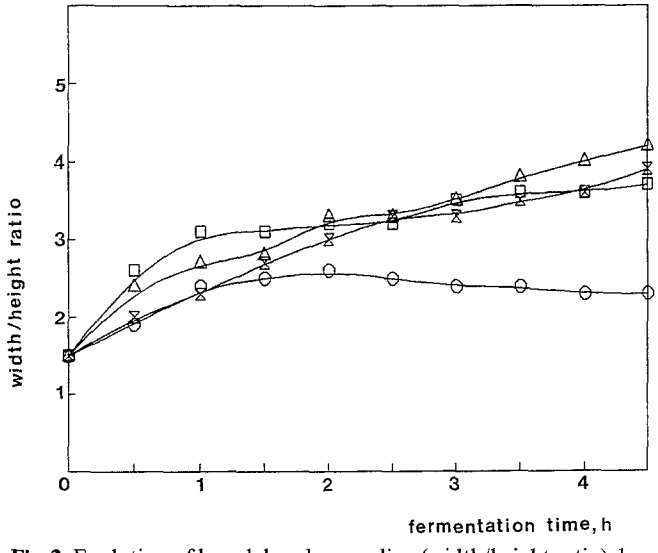

Fig. 2. Evolution of bread dough spreading (width/height ratio) during fermentation on a multistage wheat sour dough process. ⊟ BD1; ≙ BD2; ⧖ BD3; ⊖ BD5

Table 4. Evolution of rheological and fermentative characteristics of bread doughs (BD) on a multistage sour dough process

Characteristics	Bread dough stage				
	BD 1	BD 2	BD 3	BD 4	BD 5
Extensigram					
Extensibility (mm)					
30 min	177	171	158	142	131
60 min	172	160	150	118	117
90 min	160	145	131	116	109
Resistance to extension (BU[a])					
30 min	292	260	321	384	400
60 min	343	384	431	475	469
90 min	403	408	432	483	471
Maturogram					
Fermentation time (min)	127	125	125	120	117
Elasticity (BU)	98	98	113	135	173
Dough level (BU)	205	205	228	315	423
Impulsogram					
Maximum oven rise (BU)	173	155	210	205	333
Final oven rise (BU)	75	75	85	75	188

[a] Brabender units

other authors [19, 20, 35, 36]. BD stability increased (lower width/height ratio) as the number of SD stages increased. BD5 reached the maximum stability, which was maintained from 2.5 h to 6 h. However, whereas for the remaining BD, spreading values increased as fermentation time increased. Results obtained for BD5 are of the same order as those reported by Barber et al. [36] in wheat doughs with pure lactic acid bacteria. These values were lower and thus the dough was more stable than those observed with pure yeasts [36]. The effect was similar to that shown when organic acids were added to the dough [20] and in this case could be attributed to the acidifying action of the lactic acid bacteria.

3.2 Extensigram characteristics

The extensibility of BD decreased and resistance to extension increased with fermentation time (Table 4), showing trends outlined with other fermented doughs [37, 38]. With BD from each stage, similar tendencies were observed but of different magnitudes. Generally, the later stages (BD4, BD5) led to a decrease in the extensibility and an increase in the resistance of the doughs which were greater than with the BD from the first stages. These changes have been associated with higher H^+ concentrations [9, 10], with BD4 and BD5 that contained larger amounts of acids (Fig. 1), and as a consequence a lower pH. However, this confirms the improving effect of mature sour doughs on the dough's rheology as pointed out previously [1].

3.3 Maturogram and oven rise recorder

BD1 and BD2 gave similar fermentation times (125 min), elasticity (98 Brabender units, BU) and dough level (205 BU) (Table 4). The last two parameters were modified in the next BD stages. An improving effect on BD was recorded as the SD ripened. Dough elasticity increased from 113 BU (BD3) to 173 BU (BD5), and dough levels from 228 BU (BD3) to 423 BU (BD5). This effect could be related to the development of a more active and adapted microflora, as with the formation of more available substrates resulting from the prolonged action of enzymes from flour and microorganisms.

This BD behaviour was also reflected during oven rise, as recorded by the impulsograph (Table 4). A noticeable positive effect of SD stages on oven spring was observed during heating. This could be related to the increasing microflora in SD that became stabilized in SD3. BD3 and BD4 gave close values for oven spring (maximum oven volume) and final oven rise (205–210 BU and 75–85 BU, respectively); both FBD had acetic and lactic acid contents of the same order (Fig. 1) so a population of lactic acid bacteria consisting mainly of homofermentative cocci [15, 16] were present. These two factors can influence the baking microbial activity and gluten physicochemical properties during baking. Finally, for BD5 a considerable improvement was obtained as compared with BD from the previous steps (335 BU and 188 BU for oven spring and final oven rise, respectively). This dough contained higher levels of acetic acid and lower levels of lactic acid than BD3 or BD4, which is in accordance with its larger amounts of heterofermentative lactobacilli.

4 Evolution of bread characteristics

The bread (B) volume rose from 173 ml to 241 ml and the density decreased from 0.30 to 0.22 g/ml as the number of SD stages in the preparation of B increased (Table 5). Values under 0.25 g/ml are high-quality indexes for this

Table 5. Evolution of bread (B) characteristics on a multistage wheat sour dough process

Characteristics	Bread stage				
	B1	B2	B3	B4	B5
Volume (ml)	173	184	196	230	241
Density (g/ml)	0.30	0.28	0.26	0.25	0.22
Texture (cm^2)	1420	475	383	363	182
Total titratable acidity (ml 0.1 N NaOH/10 g bread wet wt)	4.0	4.7	5.1	5.5	6.5

kind of wheat bread. A greater number of stages in SD preparation resulted in a softer crumb texture (measured by the maximum deformation strength), values ranging from 1420 cm^2 (B1) to 182 cm^2 (B5). As it has been shown for rheological and fermentative parameters, a noticeable improvement in both bread volume and texture was reached when using SD5. The relative proportion of acetic and lactic acid in BD5 (3.72) and B5 (3.48) (Fig. 1) and its greater gassing power (Table 4) would considerably contribute to gluten ripeness and therefore improve the bread's characteristics.

Total titratable acidity of B samples varied from 4 to 6.5 ml (0.1 N NaOH/10 g bread wet wt.) as shown in Table 4; in every case, this was below maximum allowed by Spanish law for white wheat bread. The main sour breads were those made during the latter SD stages, which also had higher acidification levels [15, 16]. Acetic acid contents of bread (Fig. 1 c) oscillated from 0.072% (B1) to 0.095% (B5), whereas lactic acid levels ranged from 0.303% (B1) to 0.618% (B3) (Fig. 1 f) thus following the tendencies outlined for BD (Fig. 1 b, c, e, f). However, these amounts were lower than those obtained with FSD and FBD. During baking, some losses in organic acids, mainly acetic acid, can occur [21]. Owing to this, the FQ values of bread were somewhat higher (3.05–5.35) (Fig. 1 f) than those obtained for FSD and FBD. The highest value corresponded to B3, whereas the FQ of B5 was 3.48 a value close to those considered to be adequate enough to obtain a balanced flavour in rye and some wheat sour doughs [2, 3, 7]. The proportion of each isomer of the lactic acid in breads remained unchanged as compared with that of FBD (Table 2).

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