Z Lebensm Unters Forsch (1992) 194:442–449

Zeitschrift für Lebensmittel-Untersuchung und -Forschung

Original paper

Storage of packaged white bread

III. Effects of sour dough and addition of acids on bread characteristics *

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Received July 25, 1991

Lagerung von verpacktem Weißbrot
III. Wirkung von Sauerteig und von Säuren auf die Brotbeschaffenheit

Zusammenfassung. Es wurde die Wirkung von drei Sauerteigen, Vorteig (V), gekühltem Vorteig (GV) und Spontansauer (SS) mit und ohne Zugabe von Säuren (Citronensäure, Milchsäure, Essigsäure) auf die Beschaffenheit und Haltbarkeit von Weißbrot untersucht. Die einzelnen Brotmuster schwankten im pH-Wert (5,71–3,47) und Säuregrad (3,90–15,67 ml NaOH). Der SS, der den niedrigsten pH-Wert und höchsten Säuregrad und Milchsäure/Essigsäure-Quotienten aufwies, ergab ein Brot mit größtem Volumen, guter Krumenporung und geringstem Altbackenwerden während der Lagerung. Eine weitere Säuerung der SS-Probe durch Zugabe von Säuren ergab ein kleineres Volumen, gröbere Porung und weniger Aroma sowie schnelleres Altbackenwerden. Die Anwendung von 20% SS verzögerte die Schimmelbildung, und zwar verdoppelte sich die Haltbarkeit im Verhältnis zum direkt geführten Brot (D). Die Zugabe von organischen Säuren zu der 20%-SS-Probe verlängerte die Haltbarkeit um mehr als 30 Tage, je nach Art der Säure und ihrem Verhältnis: Citronensäure 0,12% < Citronen- plus Milchsäure 0,25% < Citronen- plus Milchsäure 0,5% = Citronen- plus Essigsäure 0,125%. Frische V- und D-Brote wiesen niedrigere Enthalpie-Werte (ΔH_g) als GV- und SS-Brote auf; in dem letzten nahm ΔH_g mit dem SS-Anteil zu. Zugabe von organischen Säuren zur 20%-SS-Probe verringerte die ΔH_g-Werte des frischen Brotes. Im Verlauf der Lagerung stiegen die ΔH_g-Werte aller Proben während der ersten fünf Tage an. Das 20%-SS-Brot erfuhr die kleinsten Veränderungen und die D- und V-Brote die höchsten.

Summary. Effects of three ferments, sponge (S), refrigerated sponge (RS) and spontaneous sour dough (SS), with and without addition of acids (citric acid, lactic acid and acetic acid) on the characteristics and shelf-life of white bread was studied. Bread samples ranked widely in pH (5.71–3.47) and total tritatable acid (TTA) (3.90–15.67 ml NaOH). The SS, having the lowest pH and highest TTA and lactic/acetic ratio gave the bread with the highest volume, good crumb grain and the lowest rate of staling during storage. Further acidification of 20% SS sample by addition of acids caused lower volume, coarser crumb grain and less typical flavour as well as faster staling. The use of 20% SS delayed the growth of mold, extending bread shelf-life twofold with respect to that of straight dough bread (D). Addition of organic acids to 20% SS formulation increased shelf-life up to more than 30 days, depending on the acid and its proportion: citric acid 0.12% < citric plus lactic 0.25% < citric plus lactic 0.5% = citric plus acetic 0.125%. Fresh S and D breads showed lower enthalpy values (ΔH_g) than RS and SS breads, increasing in the latter with percentage of SS added. Addition of organic acids to 20% SS bread formula decreased ΔH_g of fresh bread. During storage ΔH_g values of all samples increased sharply in the first five days. The 20% SS bread underwent the smallest change and the D and S breads the highest ones.

Introduction

The shelf-life of bread is limited by physicochemical changes, i.e. staling and microbiological spoilage. Numerous attempts have been made to improve the keeping quality of bread, by changing product formulation [1–3], processing [4–6] and packaging conditions [1, 3, 7, 8], but the preservation of sensorial characteristics remains unresolved. The present general trend to reduce the use of preservatives and treatments that might affect healthy attributes of food has led to attempts to improve bread quality and shelf-life through formulation with compounds naturally occurring in foods to regulate pH, a_w, etc., e.g. acetic acid and sugars, and to change dough fer-

* Presented in part at the 13th ICC Congress, Wien, Austria, May 1990. This paper is part of the doctoral thesis of B. Barber
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mentation towards favouring development of specific compounds associated with the desired characteristics and good keeping quality of bread, e.g. certain organic acids.

Positive effects of the use of sour dough (SD) on bread shelf-life have been reported both for rye bread [9] and wheat bread [4–6]. Reduction of bread pH through simple addition of acid to dough was found to have less influence on shelf-life than the use of sour dough [9]. On the other hand, a correlation between acetic and lactic acid content and bread shelf-life has been observed [5]. Other authors have also observed an influence of proof time on the changes in texture during storage of bread [10].

In previous work [6], it was postulated that the role of SD on bread quality and shelf-life should be attributed to the type and concentration of the organic acids formed during fermentation, more than to the pH and resulting acidity of the bread. A comparative study of the role on bread quality and shelf-life of the major organic acids resulting from dough fermentation, using either SD, addition of acids to dough or both, seems to be a promising way to attempt to clarify this thesis.

Materials and methods

Preparation of bread samples. A white wheat flour of the following characteristics was used: moisture content 14.9%, falling number 451 s, Farinograph absorption 57.4%, Alveograph deformation energy 212 mJ, P/L ratio 0.7. Formulations and procedures used for the preparation of three ferments (sponge, S; refrigerated sponge, RS; and spontaneous sour dough, SS) and bread doughs (BD) obtained thereof as well as the corresponding breads are described in Fig. 1 and Table 1. A total of 13 samples were prepared by formulating bread doughs with different ferments and acids (citric acid, lactic acid and acetic acid) at various concentrations (Table 1). After cooling to 30° C, bread loaves were packaged in polypropylene bags, 40-μm film thickness, and stored at +27° C.

Bread characteristics. pH and total titratable acidity (TTA) were measured according to the *„Arbeitsgemeinschaft Getreideforschung"* [11]. Lactic and acetic acid contents were determined by enzymatic methods [12]. The loaf volume was measured by rapeseed displacement. Moisture content was determined according to the AACC methods 62-05 and 44-15A [13]. Water activity of bread crumb was

Table 1. Formulation of bread samples

Ferment	Amount (%) of	Citric acid	Other	Sample
None (D)	0	0	–	D-0
		0.12	–	D-1
Sponge (S)	20	0	–	S-0
		0.12	–	S-1
Refrigerated sponge (RS)	20	0	–	RS-0
		0.12	–	RS-1
Spontaneous sour dough (SS)	10	0	–	ss-0
		0.12	–	ss-1
	20	0	–	SS-0
		0.12	–	SS-1
		0.12	Lactic 0.25	SS-1,1
		0.12	Lactic 0.50	SS-1,2
		0.12	Acetic 0.125	SS-1,3

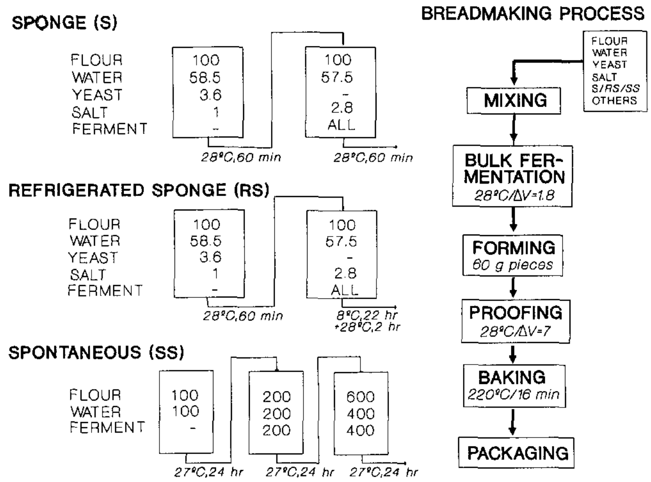

Fig. 1. Formulations and procedures for preparation of ferments and bread

determined using a thermoconstanter (Novasina). Calibration was done at 25° C using KNO_3 ($a_w = 0.925$), $BaCl_2$ ($a_w = 0.901$) and $Mg(NO_3)_2$ ($a_w = 0.529$). Sensory analysis was carried out by a panel of trained judges using semi-structured scales, scoring 0–10, of which only the extremes were described. Evaluated attributes were: appearance, aroma (intensity and characteristic), taste (intensity and characteristic) and overall acceptance. In preliminary sensory sessions, judges evaluated a reference bread (made by the straight process) and the mean of these evaluations was given as a prefixed score for the reference, judges were then asked to qualify the sample by comparing it with this reference; each judge received one loaf and one 2-cm slice of both breads.

Bread shelf-life was evaluated by direct observation of loaf crust until detection of visible mould colonies in two ten loafes sets of each sample. The end of shelf-life was established when three out of ten loaves showed mould colonies.

Crumb firmness and elasticity measurements. Crumb firmness was measured using an Instron Universal Testing Machine (UTM). The conditions used were a load cell with a maximum load of 5 kg and a crosshead speed of 100 mm/min. A 17-mm-diameter plunger compressed a 20-mm-thick slice to a depth of 75%. The height of the compression measured the resistance of the crumb to the penetrating plunger and represented the crumb firmness. A crumb cylinder of 35 mm diameter and 30 mm height was used for elastic recovery measurements. Its exact height was measured with a gauge before and after compression to 0.5 cm with the Instron UTM applying the above cited conditions and relaxation for 10 min in a plastic bag. Percentage of height recovery is used here as an index of elastic recovery of crumb. Measurements were carried out on loaves stored 0, 5, 10, 15, and 20 days at 27° C.

Differential scanning calorimetry. The instrument used was a Perkin Elmer DSC2 fitted with an integrating chart recorder model X-Y1Y2 from the same firm. Freeze-dried bread crumb (1 g) of known moisture content was intimately mixed with an appropriate amount of 0.1% propionic acid solution (to prevent moulding) in small glass bottles with air-tight lids to obtain a moisture content of 55%. After equilibration for 48 h at 6° C, portions (15–20 mg) of these sludges were transferred into preweighed aluminium DSC pans (no. 0219-0062) which were immediately sealed and the sample mass determined.

The DSC head was pre-cooled to 27° C using a cryostat. The sample pan was placed on the sample holder with a 68-mg aluminium pan as the reference. A min was allowed for equilibration of the sample chamber prior to increasing the temperature up to 117° C, at a scanning rate of 10° C/min. A steam of dry nitrogen was allowed to flush through the DSC head, at 30 ml/min, throughout the determination period. The dry matter content of each individual sample was determined after the DSC scan by puncturing and drying the pan at 100° C/vacuum for 12 h. DSC measurements were made up to 20 days, or until the bread became mouldy.

The gelatinization energy ($-\Delta H_g$) was obtained by converting the integrator response under the endotherms to energy values using a calibration coefficient previously obtained based on integrator responses for several determinations of heat of fusion of indium. For each sample the mean of three determinations was obtained. The temperature axis of the instrument was calibrated by determining the extrapolated onset temperatures of compounds of high purity and known melting point (stearic acid, benzoic acid).

Statistical analysis. The analysis of variance was applied to data, by using a Microvax (VAX/VMS) computer (BMDP 7D). Means were compared by the minimum significant difference test [14].

Results and discussion

Characteristics of ferments and bread doughs

Ferments used in preparing bread doughs differed greatly in pH, TTA and acetic acid and lactic acid contents (Table 2). Differences were minor between sponges. As expected, the SS showed lower pH and higher TTA than the sponges. Acetic and lactic acid levels found in S and RS are of similar order to those published for industrial sponges [15] used in conventional wheat flour breadmaking with relatively high percentages of yeast. The higher acid content of SS compared with S and RS is in good agreement with the predominant microflora in the former: 10^8 CFU lactic acid bacteria/g and 10^5 CFU yeasts/g vs 10^6 CFU/g and 10^8 CFU/g respectively in sponges [16] (CFU = colony-forming units).

The pH differences observed between ferments were also found among the bread doughs (BD) prepared from them. BD made with 20% S and 20% RS, like a straight dough prepared (D) in parallel showed similar pH values, while that made with 20% SS had a lower pH (Table 3). As expected, BDs with acid additions showed lower pH values, the effect being more pronounced the higher the BD pH value. Fermentation, as shown by increase of dough volume as a function of time, proceeded similarly for all BD samples without acids added. The dough with 0.125% acetic acid showed a slower rate of fermentation, as expected from the suppressing effect of this acid on the metabolic activity of bakers yeast.

Table 2. Characteristics of the ferments used to prepare bread doughs

Parameter	Value for		
	Sponge	Refrigerated sponge	Spontaneous sour dough
pH	5.4	5.5	3.7
TTA (ml NaOH)	3.66	3.59	11.45
Acetic acid	620	751	2382
D-lactic acid	42	48	5844
L-lactic acid	27	16	3207

Acid contents are given as mg/kg dry bread

Table 3. Acidity and acid content of bread samples

Sample	BD	Bread		Acid content in bread (mg/kg d.b.)		
	pH	pH	TTA	Acetic	D-lactic	L-lactic
D-0	5.29	5.71	3.90	736	71	37
D-1	4.78	4.98	5.99	–	–	–
S-0	5.33	5.50	4.16	688	66	35
S-1	4.79	5.02	5.90	–	–	–
RS-0	5.18	5.59	4.18	731	60	37
RS-1	4.77	5.07	5.58	–	–	–
ss-0	4.90	5.10	5.19	763	872	1264
ss-1	4.64	4.72	6.47	–	–	–
SS-0	4.67	4.88	6.05	817	1873	2095
SS-1	4.37	4.69	7.44	933	1406	1627
SS-1,1	4.13	4.02	11.66	741	843	3612
SS-1,2	3.77	3.47	15.67	769	828	5364
SS-1,3	4.30	4.28	9.21	1734	916	1487

– = Not determined

Characteristics of bread samples

pH and TTA levels of samples without acids added were within the typical ranges for breads made by the sponge and dough and the sour-dough processes [15] (Table 3). Addition of 0.12% citric acid to bread dough formulation lowered bread pH of D, S and RS samples to a greater extent than in the SS sample. However, the SS bread showed the lowest pH due to the much lower pH of its initial dough (Table 3). Addition of 0.12% citric acid plus 0.25% and 0.50% lactic acid to doughs formulated with 20% SS, decreased pH both of fermented dough and of bread to very low levels (Table 3). Similar, although of lower magnitude, was the effect of joint addition of 0.12% citric acid and 0.125% acetic acid. The difference in pH between fermented dough and bread ranged between 0.2–0.4 when only citric acid was added; however, smaller differences were found when lactic and acetic acids were additionally incorporated.

Lactic and acetic acid content

The acetic acid content of bread was hardly affected by the type of ferment used in breadmaking, but the lactic acid content was greatly influenced (Table 3). Samples made with the sponges S and RS, as well as by the D process, had very low lactic acid content (97–108 mg/kg, dry bread) whereas breads made with SS reached levels 20 (ss-0) to 50 (SS-0) times higher (Table 3). The fermentation quotient varied from 0.09, typical of a yeast-based fermentation [17], up to 3.2 characteristic of lactic fermentation [15]. The latter value is of the same order as those observed in bread made with rye sour dough [18] and in wheat bread with commercial starters [15]. The proportion of D(−) and L(+) isomers of lactic acid depended also on the type of ferment used. While the D(−) isomer was predominant (62–66%) in breads D-0, S-0 and RS-0, the proportion of both isomers was equilibrated more in breads with SS, the L(+) isomer being slightly more abundant. The greater proportion of the D(−)

isomer in sponge breads might be associated with the high proportion of *Lactobacillus plantarum* species in dough: 83% of total lactic acid bacteria [16]. Spicher et al. [19] found three times more D(−) than L(+)-lactic acid in rye doughs inoculated with *L. plantarum*, both individually and in the presence of yeasts.

Addition of organic acids to BD containing 20% SS affected the acetic and lactic acid contents of bread not only by its additive effects but also through its regulatory influence on the dough fermentation process. Addition of 0.12% citric acid (sample SS-1) slightly increased the acetic acid and decreased the lactic acid concentrations in bread (Table 3). Additional incorporation of lactic acid (SS-1,1 and SS-1,2) had reverse effects, as was expected, although the increase in lactic acid content of bread was less than that corresponding to the amount added. Excess of acid in dough limited the production of organic acids during fermentation as shown also by pH and TTA. By adding lactic acid the proportion of D(−) and L(+) isomers varied considerably, up to 81% and 87% L(+) was found in samples SS-1,1 and SS-1,2, respectively. Addition of acetic acid (0.125%) to the SS formula with 0.12% citric acid also decreased the lactic acid content of bread.

Volume

The type of ferment influenced the volume of the resulting bread, although all samples showed an acceptable value (Table 4). Bread made with 20% SS (sample SS-0) had the highest volume whereas that with 20% RS (sample S-0) had the lowest; loss of fermentative capacity by low temperature treatment of activated yeast was to be expected. Bread volume increased by increasing the proportion of SS, although higher SS levels result in a more dense bread. In previous work [6] the use of 30% SS led to breads of lower volume than those made with 30% sponge.

Addition of acids decreased loaf volume and slice area (Table 4); the magnitude of the effect depended more on the type of the acid than on the pH of the dough. Samples with 0.12% citric acid, having a dough pH between 4.4 and 4.8, showed a loaf volume decrease in the range of 0–10%, whereas samples with additional incorporation of 0.25% lactic acid or 0.125% acetic acid, resulting in dough pH of 4.1 and 4.3 respectively, showed a loaf volume decrease of 15% and 33%, respectively. TTA values deserve similar comments. The role of pH on bread volume [20] is probably important below 4.5; however, the inhibitory effect on yeast fermentation of certain acids such as the acetic acid predominates.

The width/height ratio of the central slice of bread, related to dough stability during baking, was quite similar whichever ferment was used and ranged between 0.85 and 0.89. Addition of 0.12% citric acid had practically no affect on this ratio with the only exception of the 20% SS bread (0.87 without acid and 1.08 with acid added), probably due to its initial higher acid content. Samples with citric acid plus lactic or acetic acid also showed higher width/height ratios, indicative of lower dough stability during baking.

Moisture content and water activity of bread

Both moisture content and water activity (a_w) were independent of the ferment type, its proportion and acids added to dough. The moisture content ranged between 29.5% and 32.0%, somewhat higher than usual in this type of bread [3, 6], as expected from the increased dough hydration given to improve crumb texture. a_w in the center of crumb ranged between 0.96 and 0.97 and was independent of the ferment and acids added. It has been reported [21] that organic acids have the capability of decreasing a_w, however, neither the acid type nor the acid content showed any definite influence on a_w within the range of conditions assayed in present study.

Crumb firmness and elasticity

Bread samples without acetic or lactic acid added showed very low firmness values (within the range 49–118 mN), being independent of the type and percentage of the ferment used and the addition of citric acid. All samples exhibited a well developed, fine, soft crumb. Breads with

Table 4. Characteristics of bread samples

Sample	Volume (cm³)	Area (cm²)	*b/a*	External appearance	Flavour		Overall acceptability	Shelf-life (days)
					Typical	Intensity		
D-0	322	20.5	0.85	4.9	5.9	7.8	5.7	5
D-1	321	20.3	0.86	5.8	6.7	6.9	6.1	5
S-0	335	21.2	0.90	7.0	6.7	6.4	5.0	7
S-1	317	20.0	0.89	6.1	6.3	6.4	4.6	7
RS-0	302	22.7	0.86	6.3	6.4	6.8	5.8	9
RS-1	273	22.0	0.85	6.7	6.2	7.2	5.2	9
ss-0	345	21.6	0.88	5.7	4.8	6.0	5.0	6
ss-1	347	21.4	0.89	5.5	3.7	6.2	3.3	6
SS-0	380	23.3	0.87	6.1	5.6	6,6	5.1	9
SS-1	363	22.8	1.08	6.5	4.4	7.5	3.8	20
SS-1,1	306	20.2	1.01	6.2	1.8	8.3	1.8	25
SS-1,2	225	18.4	1.22	5.6	1.0	8.8	1.1	>30
SS-1,3	242	17.2	1.07	6.1	2.4	7.9	2.8	>30

b/a = ratio width/height of slice

lactic or acetic acid added showed higher crumb firmness values (between 137–235 mN), in good correspondence with their lower volumes. Compression force increased with decreasing bread volume, as has been reported by other authors [10, 22, 23]. Their high acidity may be responsible for their coarser crumb.

The elastic recovery of crumb cylinders (see Materials and Methods) was a good index to evaluate the trends in crumb springiness. Recovery ratios ranged from 99.3% to 96.7%. The 20% SS bread showed the highest recovery (99.3%) followed by the 10% SS sample (97.7%), confirming the improver effect of spontaneous sour dough on crumb elasticity reported in the literature [24]. Addition of 0.12% citric acid had no influence upon the recovery ratio; however, additional incorporation of either lactic acid (0.25% and 0.50%) or acetic acid (0.125%) decreased the ratio to its lowest values (97.3%, 96.7% and 97.6%, respectively).

Sensory attributes of bread samples

Appearance. Scores ranged between 5.5 and 7.0. Qualifications were not related to any of the assayed variables (Table 4).

Flavour. Scores for taste and aroma were practically identical for each individual bread sample. Therefore both attributes are referred to together as flavour. Flavour intensity scores for breads without acids added varied within a narrow range (6.8–6.0) with no clear influence of the type of ferment used; scores for samples with 0.12% citric acid did not differ significantly from the former; the 20% SS bread was an exception (7.5 with citric acid and 6.6 without it); (Table 4). Addition of citric acid plus lactic or acetic acids increased flavour intensity. Typical flavour scores were higher for S, RS, and D breads than for SS samples. Addition of acids had no significant effect on sponge breads but decreased typical flavour in SS samples.

Overall acceptance. Scores for non-acidified samples ranged between 5.0 and 5.8; variations were not associated with the type of ferment (Table 4). Scores for samples with acids added were lower and varied within a wide range. Citric acid had little affect on the calification of sponge breads and decreased significantly the acceptance of SS breads; additional incorporation of lactic or acetic acids further decreased acceptance scores.

Effects of ferments and acids on bread storage

Bread shelf-life. Mould-free shelf-life of bread samples ranged from 5 days (sample D-0) to more than 30 days (samples SS-1,2 and SS-1,3; Table 4). S, RS, and SS breads showed a slightly longer shelf-life than D bread. Citric acid had no effect on S, RS, and D samples; it extended from 9 to 20 days the shelf-life of 20% SS bread (SS-1,0) but did not improve the 10% SS sample (ss-1). Such a remarkable effect on SS-1 bread cannot be attributed to pH (4.69) which was quite similar to that of ss-1 (4.72). In general, no correlation was found between bread shelf-life and pH. Other factors associated with dough fermentation must be involved. 0.12% citric acid plus 0.25% lactic acid (SS-1,1 sample) extended shelf-life from 9 to 25 days whereas 0.12% citric acid plus 0.125% acetic acid extended it from 9 to > 30 days, bread pH being 4.02 and 4.28 respectively. The type of acid present, with micostatic activity enhanced by the low pH, again appears to be a determinant factor.

Crumb firmness and elasticity changes during bread storage

Crumb firmness increased in all samples, changes occurring at a much greater rate in the first 5 days of storage (Fig. 2). After this period crumb firmness ranged over 8825–11 767 mN, as compared with 49–235 mN at the beginning; firmness of the 20% SS bread without acid added was close to that with citric acid. Due to the short shelf-life of other samples, only the 20% SS with acids breads were used for more extended storage studies. They differed greatly in the rate of changes; that with citric acid only underwent the slowest rate and was followed in increasing order by those with citric acid plus 0.125% acetic acid (SS-1,3) and 0.25% (SS-1,2) and 0.50% (SS-1,2) lactic acid. Crumb elasticity decreased in the first 5-day period and remained practically unchanged up to the 20th day. The 20% SS bread with citric acid changed from 98.7–99.3% at the beginning to 96.0–97.6% and those with additional lactic acid or acetic acid decreased from about 97% to 95%. Therefore, although firmness and elasticity show inverse, parallel trends, the former attribute appears to be more suitable to follow storage changes.

DSC measurements

DSC thermograms of bread crumb of all samples tested at different storage periods (0, 5, 15, and 20 days) fol-

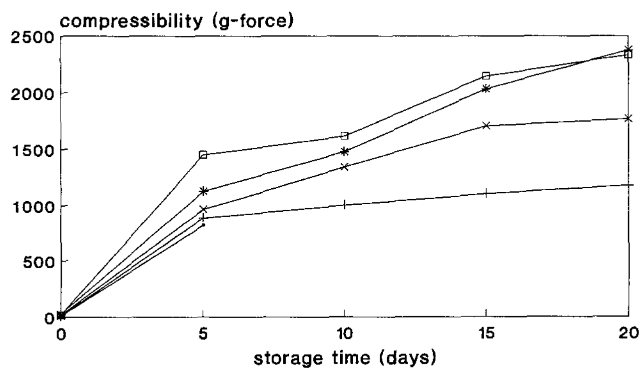

Fig. 2. Variation of crumb compressibility during storage for 20% spontaneous sour dough breads without (SS-0) and with organic acids added: citric acid (SS-1), citric plus lactic (0.25% and 0.50%) acids (SS-1,1 and SS-1,2) and citric plus acetic acids (SS-1,3). Compressibility is given as g-force: 2500 = 245 N. (–■–) SS-0; (+) SS-1; (✱) SS-1,1; (–⊟–) SS-1,2; (✕) SS-1,3

lowed the general pattern found in other studies [25, 26]. The first DSC endothermal peak (between 50° and 60° C) referred to as the staling endotherm [27] and assigned to melting of retrograded amylopectin [28], showed enthalpy values (ΔH_g) for fresh bread crumb ranging over 1.9–3.0 J/g; according to the literature, fresh bread contains practically no retrograded amylopectin [26, 29]. Although freeze-dried samples appear to give higher ΔH_g values [30] than non-lyophilized ones [26, 31], other causes, such as the absence of shortening and the relatively low moisture content, might contribute to the high values found.

Fresh S and D breads showed significantly (99%) lower ΔH_g values than RS and SS breads increasing in the latter with percentage of SS added (Table 5). Enthalpy differences indicate a higher crystallinity in RS and SS fresh breads. In the SS breads it is thought that the amount and type of organic acids present contribute to an increased retrogradation rate as a consequence of both mild hydrolysis of starch [32] and partial inactivation of α-amylases [29] during fermentation and baking. Addition of organic acids to 20% SS bread formula decreased ΔH_g values of fresh bread samples, changes being small with citric acid (SS-1) and with citric plus acetic acids (SS-1,3) and noticeable with citric plus lactic acids (SS-1,1 and SS-1,2; Table 5). The decrease of ΔH_g is attributed to an increased degree of acid hydrolysis of starch and formation of dextrins of lower molecular mass with less tendency to retrograde [29]. The effects of acid hydrolysis mask the consequences of greater inhibition of α-amylase activity. No significant differences in either the onset temperature or the maximum were observed among all fresh samples, the latter ranging over 57.5 and 58.5° C (Table 5).

During storage ΔH_g values of all samples increased sharply in the first five days. The 20% SS bread underwent the smallest change (27%) and the D and S breads the highest one (81% and 89% respectively; Table 5). Addition of organic acids to 20% SS bread formula brought about greater storage changes, in the order of 50–57%. Storage of these samples up to 20 days further increased ΔH_g with the exception of bread with citric plus

Table 5. Differential scanning calorimetric data for melting amylopectin crystallites and for dissociation of amylose-lipid complexes in the crumb of bread stored for 0, 5, 10, 15 and 20 days. t_0 = onset temperature, t_p = pasting temperature and t_c = crystallisation temperature

Sample	Storage time (days)	1st endothermic peak					2nd endothermic peak		
		$-\Delta H_g$ (J/g)	$s_{\Delta H_g}$ (J/g)	t_0 (°C)	t_p (°C)	t_c (°C)	$-\Delta H_{II}$ (J/g)	$s_{\Delta H_{II}}$ (J/g)	t_p (°C)
D-0	0	2.03	0.08	40.7	58.5	73.3	2.77	0.21	107.0
	5	3.58	0.12	42.3	58.3	78.3	3.00	0.58	106.8
S-0	0	1.91	0.21	42.2	58.2	69.2	2.96	0.25	106.5
	5	3.62	0.08	44.5	58.0	79.0	2.97	0.46	107.5
RS-0	0	3.00	0.12	38.0	57.0	73.7	2.70	0.25	107.2
	1	2.90	0.12	38.7	57.2	77.3	3.15	0.17	105.8
	5	3.00	0.12	39.5	56.5	73.2	2.78	0.17	106.8
ss-0	0	2.58	0.04	40.7	57.7	70.5	2.16	0.29	104.8
	1	2.61	0.17	43.0	58.3	76.5	2.70	0.21	105.7
	5	3.80	0.21	43.8	58.0	77.2	3.19	0.33	106.3
SS-0	0	2.80	0.21	41.2	58.3	77.2	3.21	0.21	105.8
	1	3.16	0.29	40.2	58.0	77.7	3.13	0.67	105.3
	5	3.56	0.12	41.7	58.0	77.7	2.58	0.25	106.0
SS-1	0	2.50	0.12	41.0	58.3	73.7	3.22	0.12	105.7
	1	2.98	0.04	40.3	57.8	75.5	3.39	0.17	104.5
	5	3.93	0.21	40.0	56.8	78.0	3.23	0.21	105.7
	15	4.28	0.21	38.8	56.3	80.7	3.01	0.54	107.0
	20	4.52	0.12	38.0	55.5	78.3	3.39	0.54	105.5
SS-1,1	0	2.26	0.17	40.8	58.2	71.2	3.26	0.21	105.5
	1	3.16	0.25	40.2	57.8	77.8	3.59	0.67	105.7
	5	3.49	0.21	39.0	56.8	75.8	3.93	0.50	106.0
	10	3.88	0.12	40.3	57.5	73.2	3.92	0.88	106.3
	15	3.93	0.29	41.7	56.3	73.8	4.21	0.25	106.8
	20	4.14	0.12	37.7	55.0	78.7	3.17	0.25	105.7
SS-1,2	0	2.23	0.04	39.9	58.7	72.0	3.35	0.25	106.6
	1	2.72	0.12	42.7	58.2	74.3	3.92	0.50	106.2
	5	3.41	0.12	44.0	58.5	77.0	3.36	0.17	107.5
	10	3.35	0.29	40.8	57.0	76.8	3.66	0.54	106.0
	15	3.34	0.08	44.3	58.8	75.3	3.44	0.69	107.8
	20	3.55	0.17	45.3	58.5	77.0	2.92	0.96	108.3
SS-1,3	0	2.44	0.25	43.8	58.3	72.5	2.98	0.12	106.2
	1	2.94	0.25	39.2	58.0	77.0	3.03	0.29	106.0
	5	3.67	0.04	37.8	57.0	77.8	3.00	0.12	105.3
	10	3.79	0.08	43.5	58.2	77.7	3.27	0.38	105.7
	15	4.20	0.29	40.7	57.2	77.0	3.10	0.42	106.3
	20	4.53	0.04	36.5	53.3	75.7	3.40	0.04	104.7

ΔH is given in d. b.

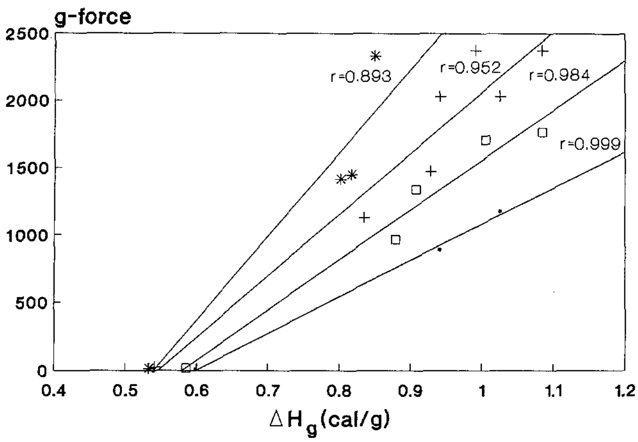

Fig. 3. Correlation between compression force and enthalpy of the amylopectin peak in bread samples formulated with 20% spontaneous sour dough and acids added: citric acid (SS-1), citric plus lactic (0.25% and 0.50%) acids (SS-1,1 and SS-1,2) and citric plus acetic acids (SS-1,3). (-■-) SS-1; (+) SS-1,1; (*) SS-1,2; (-⊟-) SS-1,3

lactic (0.50%) acids (SS-1,2) which showed almost no change (Table 5). Pasting temperature decreased with storage, reaching values between 54° C and 56° C except for the sample with highest lactic acid dose, which remained constant (Table 5). Results indicate a lower rate of amylopectin retrogradation in 20% SS breads during storage. The amylopectin fraction hydrolyzed retrograded faster in the first ten hours, decreasing the amount of starch components available for crystallization during further storage and resulting in a decreased staling rate. A good correlation between the rate of starch retrogradation and the rate of firming of bread crumb could be observed in 20% SS breads with acids (Fig. 3). Such a correlation was found by previous authors [26], while in other cases no correlation was observed [25, 33, 34]. In fact, results of compression are dependent on specific loaf volume while DSC measures changes occurring at a molecular level irrespective of macroscopic properties of bread.

No significative differences were observed for values of enthalpy of dissociation of the amylose-lipid complex either among different samples or as a function of storage time. It is noticeable that these values showed a much higher standard deviation than those of enthalpy of gelatinization. Other authors have also reported that both the number and organization of the amylose-lipid complexes are not changed during bread staling [31]. These complexes are probably formed during or immediately after baking.

Conclusions

The influence of the degree of acidity of the dough on bread quality and storage behaviour is highly dependent on its origin (use of ferments or addition of organic acids), indicating that other dough parameters besides pH, TTA and lactic/acetic acid ratio are involved.

Among the ferments assayed, the spontaneous sour dough (used in 20% proportion, flour basis) having the lowest pH and highest TTA and lactic/acetic ratio, proved to be the most favourable for obtaining a bread with the highest volume, good crumb grain and the lowest rate of staling during storage, as has traditionally been claimed [35]. The SS bread also showed a particularly characteristic flavour and a firmer crumb texture. The former is attributed to the development of aroma precursor compounds, such as organic acids [36], peptides [37], etc., and the latter resulted from incipient mild starch hydrolysis as has been mentioned, both of them being promoted by the amount and type of organic acids and overall acidity produced by sour dough microflora during fermentation.

The use of 20% SS in the breadmaking process delayed the growth of mold formation, extending twofold the bread shelf-life. Addition of organic acids to 20% SS bread formulation increased the mold-free shelf-life up to more than 30 days, depending on the acid and its proportion, citric acid 0.12% < citric plus lactic (0.25%) acids < citric plus lactic (0.50%) acids = citric plus acetic (0.125%) acids, paralleled. The order paralleled the pH decrease and TTA increase, except for the bread with acetic acid whose micostatic effect contributes additionally to extend shelf-life. The further acidification of 20% SS formula by addition of organic acids caused some impairment of bread quality (lower volume, coarser crumb grain and typical less flavour) as well as a more rapid staling. The effects of these acidifying agents on bread quality are the result of the low pH influence on yeast activity (lower CO_2 production), and on the gluten proteins (higher protein solubilization and so lower CO_2 retention) [18]. The variation of the rate of amylopectin retrogradation during storage among breads is related to the degree of acid hydrolysis of starch. Bread containing the highest dose of lactic acid and having the lowest pH and highest TTA showed a slower staling after for 5 days and storage up to 20 days, accordance with its higher level of dextrin formation. There is a significant correlation between pH, TTA and lactic/acetic acid ratio values and bread quality and storage behaviour within breads made by the same breadmaking process, which is not significant when comparing breads made by different processes.

Acknowledgement. This work was financed in part by *Comisión Interministerial de Ciencia y Tecnología* (CICYT), Spain.

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