Z Lebensm Unters Forsch A (1999) 208:394–399

ORIGINAL PAPER

M.J. Gil · M.J. Callejo · G. Rodríguez · M.V. Ruiz

Keeping qualities of white pan bread upon storage: effect of selected enzymes on bread firmness and elasticity

Received: 10 August 1998

Abstract Several different types of enzymes and their blends were added to dough with the object of improving the shelf-life of white, lidded-pan bread during storage. Bread firmness and elasticity were determined at 24, 48 and 72 h to determine the influence of the enzymes. Addition of bacterial a-amylase, specially to blends of enzymes also containing lipase and pentosanase, improved white, lidded-pan bread quality by increasing elasticity and lowering firmness of crumb, and enhanced the keeping quality over time by providing a significant 2-day increase in the shelf life.

Key words White pan bread · Enzymes · Staling · Firmness · Elasticity

Abbreviations *FAU* Fungal Amylase Units · *FXU* Fungal Xylanase Units · *MANU* Maltogenic Amylase Novo Units · *KLU* Kilo Lipase Units

Introduction

Staling of bread is usually defined as the complex changes (other than microbial deterioration) that take place after baking and which result in loss of freshness and quality of the baked product. The most important change associated with staling of white pan bread is the gradual increase in the firmness of bread crumb. The term "firmness" in a textual context refers to the force necessary to attain a given deformation. For many years, the firming of the bread crumb was thought to involve changes in the starch fraction of the flour. In particular, the recrystallization of the branched amylopectin fraction of the starch was thought to be responsible for crumb firming [1]. However, various authors [2–5] argued that starch recrystallization and bread firming are not synonymous. More recent studies [6, 7] have suggested that changes involving non-starch flour fractions such as gluten, non-starch polysaccharides, lipids and water may be important as well. The enzymes that modify both starch and non-starch flour fractions are able to influence the staling of bread. But it is important to realise that effective antistaling aspects can be considered to be specific only when they affect the increase in crumb firmness in a way independent of loaf volume, because both firmness and firming rates decrease in a linear manner with higher loaf volumes [8, 9]. Even when the volume of the bread is controlled by baking the bread in a lidded pan, antistaling enzymes still show a significant antifirming effect.

That amyloses can function as antistaling agents has been known for decades, although the specific mechanism by which amyloses retard staling are debatable. Bacterial a-amylases were the first to be recognised as exerting an antistaling effect [10–14], by hydrolysing glycosidic linkages within the amorphous areas of gelatinised starch. These enzymes reduce the staling rate while having only a minor effect on the initial softness [15]. The use of intermediate-stability bacterial a-amylases provides an antistaling effect without production of excessive levels of soluble dextrins, which can adversely affect product quality.

The effect of pentosanases on bread quality was first studied by Kulp [16]; he showed that the loaf volumes obtained after introduction of enzymes were generally slightly higher than those of the controls. Latter publications [17–20] describing the effects of added hemicellulase (specifically endo-1,4-b-d-xylanase) presented similar results, although in most cases the improvement loaf volume in was more pronounced than that reported by Kulp. Pyler [21] reported that the incorporation of pentosanases resulted in an increased loaf volume. So, the presence of hemicellulase-degrading enzymes has been shown to influence loaf volume and, consequently, crumb softness, but not the crumb staling rate.

M.J. Gil (✉) · M.J. Callejo · G. Rodríguez · M.V. Ruiz
Departamento Tecnologia de Alimentos,
Escuela Tecnica, Superior Ingenieros Agronomos,
Cuidad Universitaria, E-28040 Madrid, Spain

Lipases have been rarely used in bread making because of detrimental effects being observed due to the action of endogenous lipases liberating unsaturated free fatty acids into the dough [21]. However, beneficial effects of added enzymes on bread quality have been reported [22, 23]. Lipases can produce mono- and diglycerides from added lipids, which improve crumb softness of bread [24–27]. Addition of specific lipases in combination with triglycerides also improves loaf volume, crumb softness, and staling rate.

In addition to increased firmness, the deterioration of other textural characteristics during staling includes a considerable loss in the elasticity of the bread crumb [28–32]. The term "elasticity" refers to the ability of the bread to return to its original dimensions after being compressed. Elasticity is correlated with bread crumb structure, especially cell wall rigidity [32]. Various authors [29–36] measured elasticity as percentage recoverable work ($\%W_R$) to show that bread crumb lost its elastic properties upon staling. It has also been shown [32] that the loss in elasticity of the bread during ageing is contributed to by changes in the amorphous, continuous gluten matrix. Unfortunately, little is known about ingredients that can improve the elastic properties of bread that can be measured quantitatively.

Mechanical measurement of staling

The firmness of bread crumb can be quantified by compressing bread slices and measuring the force necessary to attain a predetermined penetration [37–40]. As the degree of staling increases, the force required for compression increases and a relationship between firmness and storage time is easily developed.

The elasticity of bread crumb can be quantified by the $\%W_R$, determined in a compression- (until predetermined penetration) decompression (at the same speed until the starting point of compression) test [9, 29–34], as the ratio:

$\%W_R$ = area under the decompression curve × 100/ area under the compression curve.

The area under the compression curve is defined as total work (W_T) the area under the decompression curve is defined as recoverable work (W_R). The loss of elasticity that accompanies bread staling is clearly manifested by the decrease in the $\%W_R$ [9, 29–32].

Accordingly, the influence of enzymes on bread quality and staling rate can be evaluated by adding different types of enzymes or blends of them to the dough to be baked into bread.

Variations in the characteristics of the bread can be evaluated using a compression-decompression test, by recording the force needed to attain the desired deformation and, also, the ratio $\%W_r$ until this deformation is achieved, in bread samples stored for 24, 48 and 72 h.

The objective of this work was to apply compression-decompression tests to study the influence of several different types of enzymes and their blends on the quality and staling rate of white, lidded-pan bread, as expressed in terms of firmness and elasticity.

Materials and methods

Experimental design

Materials. Wheat flour (13.1% protein, 14.8% moisture, 0.56% ash, 58.20% water absorption, 378 s falling number, 235×10^4 J energy of deformation, 0.48 curve configuration ratio). The rest of the ingredients were added on a percentage flour-weight basis: 2% vital gluten, 60% water, 3% sugar, 2% salt, 2% fat (vegetable margarine), 3% pressed yeast, 0.02% ascorbic acid, 0.05% lactic acid, 1% soya flour, 0.9% emulsifiers.

Enzymes. The enzymes used were: Fungamyl BG, a fungal amylase with 2500 (FAU)/g; Pentopan Mono BG, a purified endo 1,4-beta-xylanase (pentosanase) with 2500 FXU/g; Novamyl, a purified maltogenic amylase with 10 000 MANN/g; Novozym 677BG, a purified 1,3 specific lipase with 50 KLU/g. All of them are produced by Novo Nordisk. The addition of these enzymes to flour was as follows: Fungamyl BG, 1 g/100 kg flour; Pentopan Mono BG, 4 g/100 kg flour; Novamyl, 7 g/100 kg flour; Novozym 677BG, 1.5 g/100 kg flour.

Mixing was carried out using a Subal model Q12 spiral mixer at 20 rpm until optimum dough development. Loaves were formed mechanically using a Subal loaf moulder. The dough was dispensed into pans ($40 \times 11 \times 11$ cm) and lidded for fermentation in a prover at 35 °C and at a relative humidity of 80% for 35 min. The pans were closed and the bread was baked in a Termopan model Chikotherm oven. The initial temperature was 250 °C for 5 min followed by a reduction to 180 °C. The baking time was 35 min.

After baking, the bread was left to stand at room temperature for 2 h and then cooled in a temperature-controlled chamber until it reached a temperature of 40 °C at the centre, at which point it was sliced by machine and bagged in plastic. The finished bread was stored at room temperature (20–25 °C) over the period of the experiment.

Two replicate bread batches were baked for each experimental set of ingredients tested. Pans were filled and baked in tandem, yielding two samples of white pan bread per batch.

Mechanical test

Bread sample firmness and elasticity were determined mechanically using a Stevens model QTS-25 texturometer.

The end slices, which are areas of greater compaction and hence are not representative, were discarded, and then successive slices were taken for testing at each of the storage times of 24, 48 and 72 h.

Measurements were recorded for three slices from each loaf, each with an average thickness of 20 mm. The number of trials performed under the experimental design (three slices from each of four bread samples) yielded 12 compression force, W_T and W_r readings for each combination of enzymes.

Characteristics of the compression-decompression tests were: predetermined penetration, 8 mm, crosshead speed, 30 mm/min; plunger diameter, 2.54 cm.

Statistical analysis of the data

The results of the compression test were analysed statistically by means of a three-way nested ANOVA at a level of significance of $P < 0.001$. This design was thought to be the most appropriate for this type of experiment, since it enabled us to estimate measurement error and the variation of breads (the nested factor) within treatments. Mean values were compared by the least significant difference test with a level of significance of $P < 0.01$.

Results and discussion

The force-time curves obtained had the characteristic sigmoidal shape similar to that of the curves published in the AACC standard method 74-09 [37] and reported by different workers [29–31].

Tables 1–5 summarise the results of the compression tests and the corresponding statistical analyses.

Overall, the different blends of enzymes, the different storage times, the interactions between them, and, also, the breads, resulted in differentiable effects on the compression force, W_T, W_R and $\%W_R$ (Table 1).

The effect of added enzymes and storage time was significant ($P<0.001$) on compression force, W_T, W_R and $\%W_R$. The effect of interaction was only significant on the compression force and W_R; this reflected the fact that the firming rate of bread changed according to the blend of enzymes, while the effects of added enzymes and storage time on W_R and $\%W_R$ were statistically unrelated. Of course, among the compression force and W_T values, a high correlation coefficient ($92n=+0.99$) was established. The effect of bread variation was only non significant on the value of $\%W_R$.

In all six types of bread, the mean compression force values increased and the mean $\%W_R$ values decreased at 48 h and 72 h as compared to the respective values at 24 h (Tables 2, 3). The variations were statistically significant not only in the control bread, but also in breads baked either with pentosanase or with a blend of pentosanase and lipase, indicating that the staling of bread

Table 1 Results of the three-way nested ANOVA. *Bread* is the nested factor. F_C Compression force (firmness); W_T total work, W_R recoverable work, $\%W_R$ percentage recoverable work (elasticity)

Source of variation	*df*	*F*-ratio				*F* (0.001)
		F_C (N)	W_T (J)	W_R (J)	$\%W_R$	
Combination of enzymes	5	32.79***	38.85***	9.89***	32.23***	4.87
Time	2	59.78***	67.23***	12.87***	27.43***	7.91
Interaction	10	4.93***	6.00***	0.88	1.99	3.64
Bread	54	5.45***	3.00***	4.38***	1.29	2.00
Residual	144					
Total	215					

*** $P<0.001$

Table 2 Mean F_C and increase in the mean F_C according to storage time. Data followed by *different small letters* indicate significant differences [$P<0.01$; least significant difference (LSD) = 0.59]. Data followed by *different capital letters* indicate significant differences ($P<0.01$; LSD = 0.83). *Values in brackets* are statistically non-significant ($P<0.01$; LSD = 0.59). For abbreviation, see Table 1

	F_C (N)				
	24 h	48 h	72 h	Δ 24–48 h	Δ 24–72 h
Control bread	2.23 a,b	3.46 d,e	4.01 e	1.23 C–E	1.78 E
Bread with pentosanase	2.47 a,b	3.48 d,e	4.03 e	1.01 B–E	1.56 D,E
Bread with pentosanase and lipase	2.37 a,b	3.18 c,d	3.74 d,e	0.81 A–D	1.37 C–E
Bread with pentosanase and bacterial α-amilase	2.14 a,b	2.44 a,b	2.72 b,c	(0.31) A,B	(0.58) A–C
Bread with pentosanase, lipase and bacterial α-amilase	2.05 b	2.24 a,b	2.34 a,b	(0.19) A,B	(0.29) A,B
Bread with lipase and bacterial α-amilase	2.11 b	2.27 a,b	2.37 a,b	(0.16) A	(0.25) A,B

Table 3 Mean $\%W_R$ and decrease in the mean $\%W_R$ according to storage time. Data followed by *different small letters* indicate significant differences ($P<0.01$; LSD = 3.65). Data followed by *different capital letters* indicate significant differences ($P<0.01$; LSD = 5.16). *Values in brackets* are statistically non-significant ($P<0.01$; LSD = 3.65). For abbreviations, see Tables 1 and 2

	$\%W_R$				
	24 h	48 h	72 h	Δ 24–48 h	Δ 24–72 h
Control bread	21.54 c–e	17.50 a,b	14.78 a	−4.04 A–D	−6.76 C,D
Bread with pentosanase	23.65 d–f	17.87 a,b	16.76 a,b	−5.78 B–D	−6.89 D
Bread with pentosanase and lipase	21.98 c–e	18.46 b,c	17.25 a,b	(−3.52) A–D	−4.73 A–D
Bread with pentosanase and bacterial α-amilase	27.54 g	26.15 f,g	24.98 e–g	(−1.38) A,B	(−2.56) A–D
Bread with pentosanase, lipase and bacterial α-amilase	25.03 e–g	23.05 d–f	23.56 d–f	(−1.98) A–D	(−1.47) A,B
Bread with lipase and bacterial α-amilase	21.81 c–e	21.56 c–e	20.12 b–d	(−0.25) A	(−1.69) A–C

Table 4 Mean W_T and increase in the mean W_T according to storage time. Data followed by *different small letters* indicate significant differences ($P<0.01$; LSD $=21.32\times10^{-2}$). Data followed by *different capital letters* indicate significant differences ($P<0.01$; LSD $=30.15\times10^{-2}$). *Values in brackets* are statistically non-significant ($P<0.01$; LSD $=21.32\times10^{-2}$). For abbreviations, see Tables 1 and 2

	W_T (10^{-2} J)				
	24 h	48 h	72 h	Δ 24–48 h	Δ 24–72 h
Control bread	74.89 a,b	121.43 c	147.01 d	46.54 B,C	72.12 C
Bread with pentosanase and bacterial α-amilase	69.04 a,b	79.79 a,b	88.86 b	(10.74) A	(19.82) A,B
Bread with pentosanase, lipase and bacterial α-amilase	67.39 a	75.22 a,b	78.67 a,b	(7.83) A	(11.28) A
Bread with lipase and bacterial α-amilase	70.85 a,b	75.66 a,b	80.37 a,b	(4.81) A	(9.53) A

Table 5 Mean W_R and increase in the mean W_R according to storage time. Data followed by *different small letters* indicate significant differences ($P<0.01$; LSD $=4.43\times10^{-2}$). Data followed by *different capital letters* indicate significant differences ($P<0.01$; LSD $=6.27\times10^{-2}$). *Values in brackets* are statistically non-significant ($P<0.01$; LSD $=4.43\times10^{-2}$). For abbreviations, see Table 1 and 2

	W_R (10^{-2} J)				
	24 h	48 h	72 h	Δ 24–48 h	Δ 24–72 h
Control bread	15.95 a	21.17 b,c	21.66 c	5.22 A	5.71 A
Bread with pentosanase and bacterial α-amilase	18.96 a–c	20.83 b,c	22.16 c	(1.86) A	(3.19) A
Bread with pentosanase, lipase and bacterial α-amilase	16.81 a,b	17.21 a,b	18.38 a–c	(0.40) A	(1.57) A
Bread with lipase and bacterial α-amilase	15.23 a	16.16 a	16.00 a	(0.93) A	(0.77) A

had taken place. Otherwise, in breads baked with blends of enzymes containing bacterial a-amylase, the variations were statistically non-significant. The results showed that the addition of such blends with bacterial a-amylase retarded the staling of white, lidded pan bread for 48 h.

The firmness and elasticity of bread crumb baked with pentosanase increased with respect to the control bread at all measurement times (Tables 2, 3). The increase in firmness with time (comparing values at 48 h and 72 h with the values at 24 h) were smaller than in the control, while the decreases in elasticity with time (also comparing values at 48 h and 72 h) were greater than in the control. But these variations with respect to the control were not statistically significant, so the addition of pentosanase had no detectable effect on the quality and staling rate of the white, lidded-pan bread. The results reported in other studies [16–21] show that added hemicellulases do not modify the crumb firming rate, but decrease crumb firmness, probably by increasing loaf volume. The fact that, in this study, the volume of bread was controlled by baking it in a lidded pan, could explain why added pentosanase did not improve bread crumb firmness.

Generally speaking, bread baked with a blend of pentosanase and lipase showed lower crumb firmness and higher crumb elasticity than both control and pentosanase-treated breads, at all measurements times (Tables 2, 3). Over time, the increase in firmness and the decrease in elasticity were less pronounced than in the control and pentosanase-treated breads. But these beneficial effects of added lipase were only apparent because there was not sufficient evidence in the data, at $P=0.01$, to conclude that the addition of lipase could improve the quality and retard the staling of white, lidded-pan bread. However, various authors have reported that the addition of lipases in combination with triglycerides improves loaf volume, crumb softness and staling rate [22–26]. Lipases can produce mono- and diglycerides from added lipids, which will improve crumb softness, although it should be realised that the crumb-softening effects of lipids result in part from increased loaf volume, causing less dense and therefore softer bread crumb. It is possible that low levels of butter fat, added as substrate for lipase, and baking the bread in a lidded pan in order to control bread volume, decreased the effects of lipase in this work.

As expected, breads baked with blends of enzymes containing bacterial a-amylases showed lower crumb firmness and higher crumb elasticity than both control pentosanase-treated breads at all measurements times (Tables 2, 3). The variation was always statistically significant at 48 h and 72 h and only occasionally significant at 24 h in breads baked with a blend of pentosanase and bacterial a-amylase, for the elasticity of crumb; the decrease firmness and the increase in elasticity were more pronounced, but not statistically different, in the presence of lipase and pentosanase respectively. The increase in crumb firmness with storage time was always significantly smaller in breads baked with blends of enzymes containing bacterial a-amylase than in the control (when comparing values at 48 h and 72 h with those (at 24 h) and pentosanase-treated breads (only when comparing values at 72 h with those

at 24 h). But the decrease in crumb elasticity with storage time, was only significantly smaller in breads baked with a blend of enzymes containing pentosanase and lipase in addition to bacterial a-amylase, than in the control and pentosanase, containing breads (when comparing values at 72 h with those at 24 h). The results showed that the addition of bacterial a-amylase, specially to breads also containing pentosanase and lipase, improved quality and retarded the staling of white, lidded-pan bread. Similar results about crumb firmness and firming rate have been reported in other studies [10–14]. In this study, bacterial a-amylase, that primarily affects only amylopectin and therefore reduces the firming rate (staling), also decreased the bread's loss of recoverability (elasticity) with staling (Tables 4, 5). This is caused (32) by changes in the amorphous components, such as gluten, rather than amylopectin crystallisation.

In the control bread, the increases in both W_T and W_R with storage time were always statistically significant, and the increase in W_T was the most pronounced. This resulted in a significant decrease in $\%W_R$ (elasticity) with time. On the other hand, in bacterial-a-amylase-containing breads, these variations were statistically non-significant and, because of this, the variation of $\%W_R$ (elasticity) with time was also non-significant. In general, breads with bacterial a-amylase, showed lower W_T and W_R than control bread, but only the variation of W_T at 48 h and 72 h was statistically significant. This resulted in a significant decrease in $\%W_R$ (elasticity) at these measurement times. So, the addition of bacterial a-amylase to blends also containing pentosanase and lipase reduced the amount of $\%W_R$ (elasticity) lost upon bread staling, by decreasing the bread W_T, and had little or no effect on W_R. Probably, the effect on W_T resulted from a high correlation coefficient between this parameter and the compression force, while W_R should have been related to the amorphous components.

Variation in the compression force and $\%W_R$ for each bread type with respect to the values measured at 24 h for the control bread provides a basis for quantifying alterations in each bread type and the improvement in bread quality achieved by the addition of enzymic blends. This variation was statistically significant for breads baked either with pentosanase or with a blend of pentosanase and lipase, and non-significant for breads baked with blends of enzymes containing bacterial a-amylase (Tables 2, 3). As a result, the bacterial-a-amylase-treated breads exhibited the same firmness and elasticity after 3 days of storage as the control bread did on day 1.

The following conclusions may be drawn from the results of the mechanical measurements taken under the experimental conditions and the statistical analysis of the data.

1. Staling of white, lidded-pan bread may be evaluated on the basis of the increase in firmness values and the decrease in elasticity values, measured using a compression test.
2. Addition of either pentosanase or a blend of pentosanase and lipase to dough did not have a clear, positive effect on the quality and staling rate of white, lidded-pan bread.
3. Addition of bacterial a-amylase, specially to blends of enzymes also containing lipase and pentosanase, improved the quality of white, lidded-pan bread by increasing the elasticity and lowering the firmness of crumb, from the second day of storage, and enhanced the keeping quality over time by providing a significant 2-day increase inthe shelf life.

References

1. Pyler EJ (1973) Baking: science and technology. Siebel, Chicago
2. Dragsdorf RD Varriano-Marston E (1980) Cereal Chem 57:310–314
3. Ghiasi K, Hoseney RC, Zeleznak K, Rogers DE (1984) Cereal Chem 61:281–285
4. Pomeranz Y, Meyer D, Seibel W (1984) Cereal Chem 61:53–59
5. Rogers DE, Zelezniak KJ, Lai CS, Hoseney RC (1988) Cereal Chem 65:398–401
6. Martin ML, Zeleznak KJ, Hoseney RC (1991) Cereal Chem 68:498–503
7. Martin ML, Hoseney RC (1991) Cereal Chem 68:503–507
8. Axford DWE, Colwell KH, Cornford SJ, Elton GAH (1968) J Sci Food Agric 19:95
9. Ponte HJG, Titcomb ST, Cotton RH (1962) Cereal Chem 39:437
10. Hebeda RE, Bowles LK, Tegue WM (1991) Cereal Foods World 36:619–624
11. Oleson T (1991) Antistaling process and agent. Novo Nordisk patent no. WO 9104669
12. Akers AA, Hoseney RC (1994) Cereal Chem 71:223–226
13. Gerritsen WJ (1995) Voeding 56:26–27
14. Si QJ (1996) Food Tech Int Europe Annual, pp 60–64
15. Si QJ, Simonsen R (1994) In: Proceedings of the International AACC/ICC/CCOA Symposium, Beijing, November
16. Kulp K (1968) Cereal Chem 45:339
17. Krishnarau L, Hoseney RC (1994) J Food Sci 59:1251
18. Rouau X, Moreau D (1993) Cereal Chem 70:626
19. Rouau X, El- Hayek ML, Moreau D (1994) J Cereal Sci 19:259
20. Gruppen H, Kormelink FJM, Voragen AGJ (1993) J Cereal Sci 18:129
21. Pyler EJ (1988) Baking: science and technology, 3rd edn, vol 1. Sosland, Merrian
22. Johnson RH, Welch EA (1968) US patent no. 3, 368, 903
23. Mohsen SM, Alian AM, El-Azhary T (1986) Egypt J Food Sci 14:175–182
24. Krog N, Jensen BN (1970) J Food Technol 5:77–87
25. Krog N (1971) Starch 23:206
26. Krog N, Olesen SK, Toernaes H, Joensson T (1989) Cereal Foods World 34:281
27. Si QJ, Hoseney RC (1994) In: Proceedings of the International AACC/ICC/CCOA Symposium, Beijing, November
28. D'Appolonia BL, Morad MM (1981) Cereal Chem 58:186–190
29. Kou Y, Chinachoti P (1991) Cereal Foods World 36:888–892
30. Nussinovitch A, Steffens MS, Chinachoti P, Peleg M (1992) J Texture Stud 23:13–24
31. Nussinovitch A, Steffens MS, Chinachoti P (1992) Cereal Chem 69:678–681

32. Rao PA, Nussinovitch A, Chinachoti P (1992) Cereal Chem 69:613–618
33. Olkku JE, Sherman P (1979) Food Texture Rheol:157–175
34. Lee YC, Rosenau JR, Peleg M (1983) J Texture Stud 14:143–148
35. Kaletunc G, Normand MD, Nussinovitch A, Peleg M (1991) Food Hydrocol 5:237–247
36. Kaletunc G, Normand MD, Jonhson EA, Peleg M (1991) J Food Sci 56:950–953
37. AACC (1983) Approved methods of the American Association of Cereal Chemists: methods 79-09. American Association of Cereal Chemists, St Paul, Minn
38. Abu-shakra MA, Sherman P (1984) Rheol Acta 23:446–450
39. Bourne MC (1993) Texture measurements of finished baked goods. In: Kamel BS, Stauffer CE (eds) Advances in baking technology. Blackie, New York, pp 134–150
40. Gil MJ, Callejo MJ, Rodríguez G (1997) Z Lebensm Unters Forsch A 205:268–273