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Utilization of Enzyme Mixtures To Retard Bread Crumb Firming

ALBERTO E. LEÓN,*,† ENCARNA DURÁN,‡ AND CARMEN BENEDITO DE BARBER‡

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), P.O. Box 73, 46100 Burjassot, Valencia, Spain, and Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, CC 509, 5000 Córdoba, Argentina

The influence of enzyme mixtures containing amylase and lipase activities on straight dough bread staling was studied. Amylopectin retrogradation, crumb firming, amylose−lipid complexes, and dextrin production were analyzed in bread samples supplemented with two enzyme mixtures. The addition of enzyme mixtures to bread formula causes a beneficial effect on bread keeping properties and the formation of a more thermostable amylose−lipid complex than the one found in control bread. Amylopectin retrogradation was inhibited by the use of the enzyme; the effect was accompanied by reduced crumb-firming rates. The enzymatically generated water-soluble dextrins (maltose and DP3, DP4, DP5, and DP6 dextrins) are the most effective in preserving crumb softness during bread storage.

KEYWORDS: Bread staling; amylopectin retrogradation; dextrin; α-amylase; lipase

INTRODUCTION

Staling can be defined as the group of changes, other than the ones caused by spoilage microorganisms, which take place during bread storage and make the product less acceptable to consumers (*1*). This process involves different physicochemical transformations. However, the firming of crumb with time is the most used parameter to follow bread staling during storage (*2*, *3*).

Schoch and French (*4*) attributed bread firming to changes in amylopectin within the starch granules. However, bread firming and starch retrogradation are not synonymous, suggesting that other factors are involved (*5*−*12*). Despite the known facts, the staling mechanism is still a matter of debate.

Several additives are utilized in bread formulas as shelf-life improvers to inhibit physical changes that lead to crumb firming. Surfactants and enzymes are the ones mostly employed for this purpose. Amylose−surfactant complexation is part of the explanation for the antifirming effect of commercial emulsifiers. These complexes may prevent amylose/amylopectin retrogradation or lead to fewer nuclei that could promote amylopectin retrogradation and the development of a crystalline structure (*13*).

Among the enzymes added to bread recipes, α-amylases are the more commonly used. Silberstein (*14*) attributed the softening effect of bacterial α-amylases to a reduction of the starch level available to retrograde (by degrading part of the starch to dextrins), whereas Martin and Hoseney (*11*) assigned its effect to starch−protein or protein−protein entanglement interference by low molecular weight dextrins. Besides, lipases can be used to retard bread staling by forming monoglycerides, which act as surfactants (*15*).

Dextrins produced by enzymatic hydrolysis of starch interfere with amylopectin retrogradation (*16*−*21*).

The objective of this work was to study enzyme mixtures' influence over the staling of bread in order to contribute to a better understanding of how these enzymes exert their beneficial effect on bread keeping properties during storage. With this aim, amylopectin retrogradation, amylose−lipid complexes, and dextrin production were analyzed together with their relationship with crumb firming rate during bread storage.

MATERIALS AND METHODS

Enzyme Mixtures. Enzyme mixture a, EMA [Stalingase B (Gist-Brocades Food Ingredients Division)], is a commercial blend of enzymes obtained from strains of *Bacillus subtilis*, fungi of *Aspergillus* species, and *Rhizopus arrhizus*, containing fungal and bacterial α-amylase and lipase activities (10000 SKB/g; 1000 LU/g, approximately).

Enzyme mixture l, EML [Stalingase L (Gist-Brocades Food Ingredients Division)], is a commercial blend of enzymes obtained from particular strains of *Aspergillus* species and *R. arrhizus*, containing lipase and α-amylase as principal and secondary activities, respectively (5000 LU/g; 3000 SKB/g, approximately).

Bread-Making Process. Bread samples were made by straight dough procedure using the following baking formula:

Flour (protein = 12.4% and ash = 0.6%) (3000 g), 60.0 g of salt, 30.0 g of shortening, 6.0 g of sodium propionate, 120 g of comprised yeast, and 1740 mL of water were optimally mixed and fermented for 60 min at 28 °C and 80% moisture. After degassing, the dough was divided in pieces that were proofed for 90 min at 28 °C and 80% moisture. Baking was achieved in an electric oven at 180 °C for 18 min.

This recipe was baked with no addition of enzyme mixtures (control bread) and with the addition of 0.03% of EMA and 0.03% of EML. All bread samples were made in duplicate.

* Author to whom correspondence should be addressed (telephone 54-351-4334116/17; fax 54-351-4334118; e-mail aeleon@agro.uncor.edu).
† Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba.
‡ Instituto de Agroquímica y Tecnología de Alimentos.

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Table 1. Bread Characteristics

bread[a]	vol (cm^3)	wt (g)	specific vol ($cm^3\ g^{-1}$)	sensory evaluation 0 h	sensory evaluation 48 h
c	1262 ± 16	233.1 ± 4.5	5.41 ± 0.11	5.79 ± 0.33	3.27 ± 0.38
a	1456 ± 19	252.7 ± 5.3	5.76 ± 0.13	6.29 ± 0.39	4.78 ± 0.43
l	1435 ± 23	234.5 ± 5.7	6.12 ± 0.21	6.77 ± 0.23	5.35 ± 0.32

[a] c, control bread; a, bread supplemented with EMA; l, bread supplemented with EML.

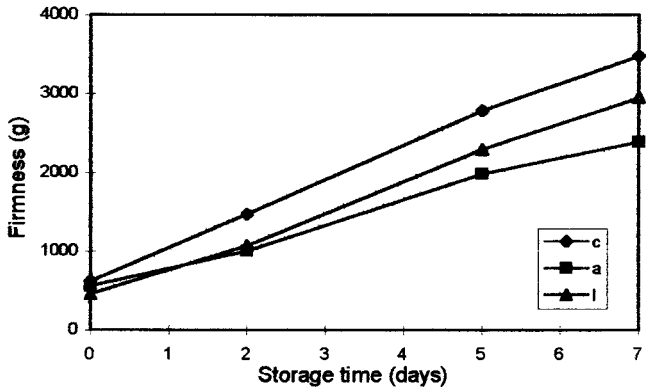

Figure 1. Effect of enzyme addition on crumb firming rate (c, control bread; a, bread supplemented with EMA; l, bread supplemented with EML).

Bread samples were stored in sealed polypropylene bags at 23 ± 1 °C.

Bread Characteristics. Volume of bread samples was measured by millet seed displacement, and their specific volume was calculated as volume/weight ($cm^3\ g^{-1}$).

Sensory evaluation was performed by a group of 10 trained panelists evaluating overall acceptance with fresh and 48-h-aged bread samples.

Texture Profile Analysis. A double cycle was performed in bread samples during storage in a TA-XT2i texturometer (Stable Microsystems, Surrey, U.K.) using a 25 mm diameter plunger at 1.6 mm/s. Resistance (g) of 20-mm-thick slices to 75% compression (firmness) was evaluated as well as springiness (defined as the time of second compression/time of first compression).

Differential Scanning Calorimetry (DSC). Amylopectin retrogradation and amylose−lipid complex dissociation were evaluated by DSC in all crumb bread samples at different storage times: 0, 2, 5, and 7 days. DSC measurements were in a DSC-7 (Perkin-Elmer), using stainless steel capsules (PE 0319-0218). Lyophilized samples were weighed in pans and mixed with deionized water (crumb/water ratio = 1:3). An Al_2O_3 plus water mixture was used as reference. Samples were heated from 30 to 130 °C at 10 °C/min. Two replicates of all samples were analyzed.

Analysis of Low Molecular Weight Dextrins. Water-soluble dextrins and sugars were quantified at 0, 2, 5, and 7 days by high-performance liquid chromatography (HPLC). Extraction from 2.0 g of lyophilized bread crumb was performed with 20 mL of 50% methanol. Extracts were further diluted with the same solution (lyophilized bread crumb/methanol = 1:100) and treated with 5% w/v ion exchange resin (Amberlite MB-1, Sigma Chemical Co., St. Louis, MO) to remove ash. Before injection in the chromatograph, extracts were evaporated, resuspended in water, and filtered.

An Aminex HPX-42A column (300 × 7.8 mm, particle size = 25 μm, cross-linkage = 4%, pH range 6−8) was used together with a deashing guard precolumn. The mobile phase was water at a flow rate of 0.5 mL/min, the column temperature being 85 °C. Lactose was used as internal standard. A solution composed of maltoheptaose, maltohexaose, maltopentose, maltotetraose, maltotriose, maltose, lactose, glucose, and fructose (Sigma Chemical Co.) was employed for peak identification and quantification. All analyses were performed in duplicate.

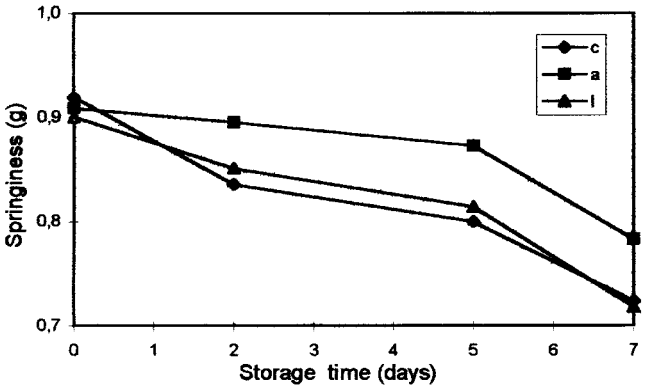

Figure 2. Effect of enzyme addition on crumb springiness (c, control bread; a, bread supplemented with EMA; l, bread supplemented with EML).

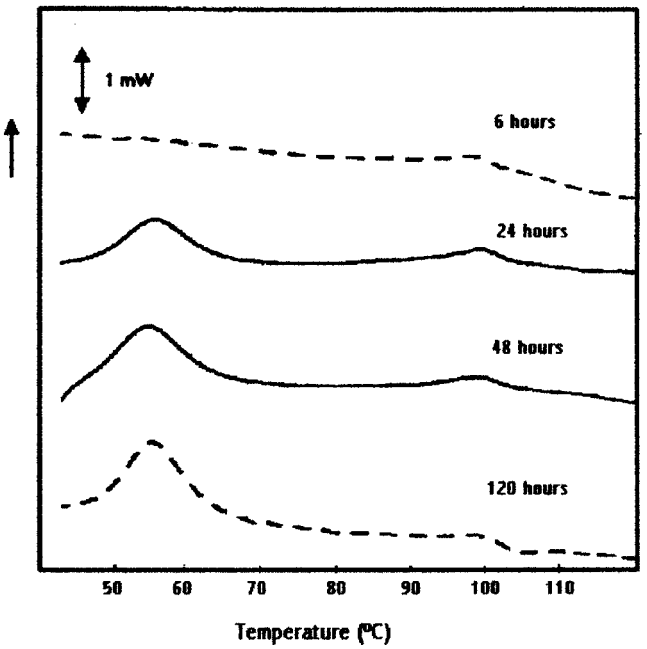

Figure 3. DSC thermograms of bread crumb at different storage times.

RESULTS AND DISCUSSION

Bread Quality. Both enzyme mixtures, EML and EMA, added to the bread formulas resulted in a product quality improvement and better acceptability by the panel when fresh bread and bread aged for 48 h were evaluated and compared to control bread (**Table 1**).

When no lipid additives are used in bread-making, lipases are known to be ineffective as bread quality improvers (*15*). However, results obtained with the addition of the EML-containing lipase and amylase activities to bread showed a strong effect despite the low content of lipid in the formulation.

Crumb Texture. During the 7 days of storage, crumb firmness linearly increased with time (**Figure 1**).

When the slope of the firming curve was analyzed, it could be observed that EML exerted a greater influence in crumb initial softness than EMA did. The latter exhibited a greater effect on product texture evolution, suggesting the different mode of action of the two enzyme mixtures used in this work. EML acted as a crumb softener and had effects similar to those of surfactants. The α-amylase activity, predominant in EMA, imparted a slower crumb firming rate rather than having an effect on initial firmness values.

Springiness (**Figure 2**) was evaluated in samples using the TA-XT2. This parameter presented a significant decay during

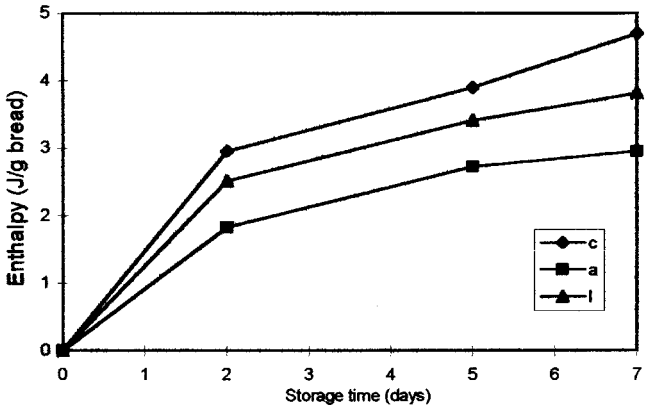

Figure 4. Amylopectin recrystallization, measured as melting enthalpy, in bread crumb during storage (c, control bread; a, bread supplemented with EMA; l, bread supplemented with EML).

Table 2. Glucose, Maltose, and Dextrin Products during Bread Crumb Storage (Milligrams per Gram of Crumb)

storage (days)	bread[a]	glucose	maltose	DP3	DP4	DP5	DP6	DP7	DP+7
0	c	26.9	94.0	5.8	0.9	1.1	1.8	3.2	51.5
	a	29.4	111.3	9.1	4.3	6.4	6.6	7.0	59.8
	l	28.0	108.6	6.8	3.0	3.8	4.4	6.2	56.2
2	c	24.6	102.0	7.7	1.8	2.0	3.1	4.1	53.0
	a	24.0	118.8	10.4	5.6	7.6	7.5	7.1	57.3
	l	27.1	119.3	8.9	4.2	6.1	6.5	7.1	51.8
5	c	22.7	115.3	7.3	2.7	3.7	4.4	4.7	51.6
	a	25.8	122.8	9.8	4.6	6.9	6.9	6.5	50.9
	l	23.5	107.1	8.4	3.6	5.7	6.2	6.5	47.6
7	c	23.3	82.6	5.4	1.5	2.1	4.0	4.3	52.0
	a	24.0	95.2	7.7	4.5	6.8	7.2	7.2	57.1
	l	24.5	87.9	7.0	3.5	5.9	7.0	7.0	52.4

[a] c, control bread; a, bread supplemented with EMA; l, bread supplemented with EML.

storage time. EML addition did not have any effect on these parameters, whereas EMA lowered springiness decay with storage time.

Starch Retrogradation. Retrograded amylopectin was analyzed by DSC, being first detected in samples of 2-day-aged bread by the endotherm named "staling endotherm" (48−67 °C). Enthalpy values (ΔH) increased with storage time (**Figure 3**). Because of lyophilized sample rehydration before calorimetry, certain retrogradation in the crumb sample may develop (*16*). To avoid the appearance of such a retrograded amylopectin endotherm in samples of fresh bread, a water/crumb ratio of 3:1 (v/w) was used, which reduced the time required by the sample to homogenize to 3 h.

ΔH values were compared among samples, as an index of starch retrogradation in bread crumb (**Figure 4**). The addition of enzyme mixtures to bread formulas caused the inhibition of starch retrogradation, with EMA being the more effective. This result was in agreement with previous studies in which bacterial α-amylase effects on retrogradation were analyzed (*16*, *17*).

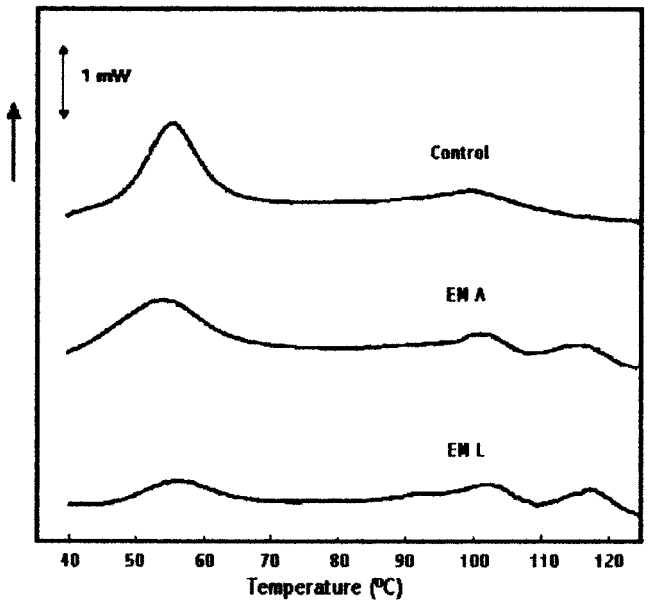

Figure 5. DSC thermograms of bread crumb.

Amylose—Lipid Complex. An amylose−lipid complex dissociation endotherm was detected when crumb samples were heated in the calorimeter from 30 to 130 °C. A second endotherm appeared in samples supplemented with enzyme mixtures and was characterized by a slightly higher peak temperature than the first one (**Figure 5**).

The combination of α-amylases and lipases may produce amylose−lipid complexes with a higher thermostability than the ones commonly determined in bread crumb. This phenomenon could partially explain bread softness found in EML-supplemented breads at the beginning of storage, when amylose retrogradation has an important role in texture.

Water-Soluble Dextrin Analysis. Dextrins appeared during the baking process as a result of α-amylase activity present in bread dough because of enzyme addition. Intermediate molecular weight dextrins were mostly found in bread crumb supplemented with EMA, which contained bacterial α-amylase. EML, containing fungal α-amylase, generated lower dextrin production than EMA did (**Table 2**).

Statistically significant differences were not found during storage when dextrins having degrees of polymerization (DP) of 4−7 were quantified, even when the sum of all dextrins was considered. Glucose, maltose, and maltotriose percentages changed during storage, probably due to changes in extractability rather than residual enzymatic activity in bread crumb.

Table 3. Correlation between Level of Enzymatic Hydrolysis Products and Crumb Firming Rate Measured with TA-XT2 (FRT)[a]

	FRT	glucose	maltose	DP3	DP4	DP5	DP6	DP7	DP+7
FRT	1.00								
glucose	–0.56	1.00							
maltose	–0.89*	0.65	1.00						
DP3	–0.96**	0.57	0.96**	1.00					
DP4	–0.98**	0.57	0.91**	0.98**	1.00				
DP5	–0.97**	0.64	0.92**	0.96**	0.99**	1.00			
DP6	–0.97**	0.66	0.90**	0.95**	0.99**	0.99**	1.00		
DP7	–0.88*	0.66	0.85*	0.89*	0.95**	0.97**	0.97**	1.00	
DP+7	–0.72	0.10	0.58	0.76	0.70	0.63	0.64	0.55	1.00

[a] * and ** indicate that the parameters are significantly or highly significantly correlated at 0.05 and 0.01 probability, respectively.

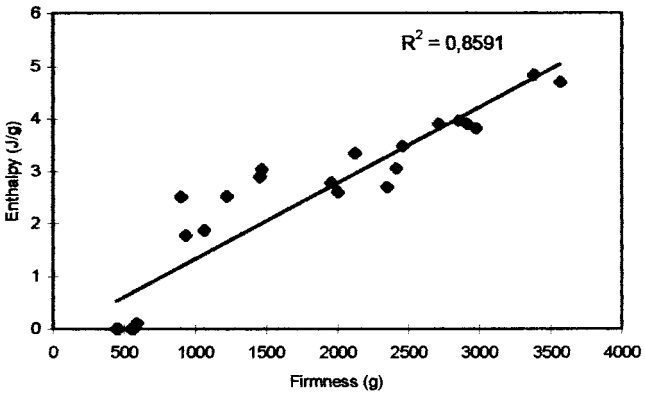

Figure 6. Relationship between amylopectin recrystallization, melting enthalpy, and crumb firming measured by TA-XT2.

Correlation Study. Quite strong relationships between bread firmness, measured by TA-XT2, and starch retrogradation occurring during bread storage were found (**Figure 6**).

Although the most important role in bread firming has previously been assigned to gluten−starch interactions (*10*, *11*), it is clear that amylopectin recrystallization is strongly associated with crumb firming rate. However, it must be emphasized that crumb firmness values continue to increase with storage time when amylopectin retrogradation reaches its maximum value and stabilizes.

Correlations among different enzymatic product contents and bread crumb firming rates were evaluated (**Table 3**). The crumb firming rate decreased with increasing dextrin contents. The best correlations were obtained with maltose, DP3, DP4, DP5, and DP6 dextrins. Similar results were obtained when starch gels were stored (*17*, *21*) and wheat flour doughs were heated in a calorimeter capsule (*16*, *18*−*20*). Because high correlation coefficients were found for the different dextrin populations, it was not possible to determine whether any of the quantified dextrins had a greater effect on crumb softness than the others.

The enzyme mixtures used acted as bread quality and bread preservation improvers in these types of bread samples.

The addition of enzyme mixtures to bread formulas caused the formation of a more thermostable amylose−lipid complex than the one found in control bread. It is unclear if this is the reason for its antifirming action at the beginning of storage time. Amylopectin retrogradation was inhibited by the use of the enzyme mixtures, and crumb firming rate was strongly related to this effect.

Maltose and the water-soluble dextrins (DP3, DP4, DP5, and DP6 dextrins) were the most effective in preserving crumb softness.

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