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ORIGINAL PAPER

Clara Bollaín · Alessandro Angioloni ·
Concepción Collar

Bread staling assessment of enzyme-supplemented pan breads by dynamic and static deformation measurements

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Abstract Dynamic mechanical analysis and thermal mechanical analysis techniques were used in order to evaluate the effect of amylolytic-, non-amylolytic- and gluten-cross-linking enzymes on the viscoelastic properties of fresh and stored pan breads. The relationships between dynamic and static compression measurements in bread staling characterization and the correlations between sensory firmness and thermomechanical properties were also investigated. Rheological changes in bread associated with recipe and storage time were successfully detected via dynamic thermomechanical analysis (DTMA) in the compression mode. Relationships between the dynamic (DTMA) and static (texture analysis) methods were found. All the bread quality indicators measured by static methods were negatively correlated with the viscoelastic dynamic parameters.

Keywords Bread staling · Dynamic mechanical analysis · Static measurements · Sensory assessment

Introduction

Food texture is one of the most important quality indicators for the acceptance of baked goods by consumers and producers. Bread rapidly loses freshness, is subject to mould spoilage and its limited shelf life has an important economical impact on the baking industry and consumers. Changes in flavour and texture taking place during storage are commonly called staling. This phenomenon, which makes the product dry and hard, is frequently attributed to starch retrogradation, which is considered as the main factor responsible for the observed increase in crumb firmness during storage [1, 2]. Although some authors state that in addition to starch retrogradation, gluten and lipids also play important roles in bread staleness [3, 4, 5].

Use of starch- and non-starch-degrading enzymes in baking provides enhancement of the fresh quality and/or inhibition of staling of bakery products [6]. Bacterial maltogenic α-amylase has been found to have both initial crumb softening and antistaling effects [4] by degradation of both amylose and amylopectin during baking. Xylanases with fewer side activities and which may be used at lower doses than traditional pentosanases act as dough-conditioning enzymes that improve dough handling and ovenspring, resulting in a bread volume increase and softer texture [7]. Microbial transglutaminase catalysed reactions modify the functional properties of baked goods via aggregation and polymerization of proteins by disulfide interchange reactions during mixing, leading to a protein network with the viscoelastic properties required for breadmaking [8, 9, 10].

Hardness is often used as a measure of bread staling, which has been determined successfully by using a texture analyser in a static compression mode [11, 12, 13, 14]. A standard method [15] for bread staling based on force–deformation for firmness in the static compression mode is available. The results obtained with this traditional instrument are limited to empirical correlations because the tests do not provide fundamental rheological data.

A bread crumb is a complex solid matrix composed mainly of gluten, starch, lipids and water, representing a typical viscoelastic biopolymer system. Frequently, the applied force is not linearly related to the corresponding deformation [16]. For that reason, it is really difficult to compare the results from many of the published reports obtained with different instruments and procedures. Dynamic mechanical analysis (DMA) and thermal mechan-

C. Bollaín · C. Collar (✉)
Cereals Laboratory, Department of Food Science,
Instituto de Agroquímica y Tecnología de Alimentos (CSIC),
Apartado de Correos 73, 46100 Burjassot, Spain
e-mail: ccollar@iata.csic.es
Tel.: +34-963-900022
Fax: +34-963-636301

A. Angioloni
Dipartimento Scienze degli Alimenti,
Corso di Laurea Scienze e Tecnologie Alimentari,
Università di Bologna,
Via Ravennate 1020, 47023 Cesena, Italy

ical analysis (TMA) have been used in order to determine dynamic rheological properties of polymeric material including foams. Recently, they have also been used for textural characterization of bakery products [12, 17, 18, 19, 20, 21]. These analyses solve the limitations of a shear rheometer employed on solid materials by operating in an oscillatory compressive mode [22]. The instrument records, at the same time, viscous (loss modulus, E'') and elastic (storage modulus, E') responses of a sample and provides fundamental rheological data. During the test, stress is applied in a sinusoidal mode to the sample and the responding strain can be in phase with the stress (if the material is an ideal elastic) or out of phase (if the material is viscoelastic). Stress and strain are out of phase by a sinusoidal angle of δ; the loss tangent, $\tan\delta$, can be calculated as the ratio between E'' and E'.

The objective of this study was to apply DMA and TMA techniques

1. To evaluate the effect of amylolytic-, non-amylolytic- and gluten-cross-linking enzymes on the viscoelastic properties of fresh and stored pan breads, as related to dynamic frequency and temperature sweep.
2. To determine the relationships between dynamic and static compression measurements in bread staling characterization.
3. To establish correlations between sensory firmness and the thermomechanical properties.

Materials and methods

Basic ingredients, additives and enzymes

Commercial blends of Spanish wheat flours (white/whole) of 13.95/13.84% moisture, 0.66/1.33% ash content, 14.08/15.31% protein, 92/61 gluten index and Chopin Alveograph parameters: energy of deformation 308×10^{-4} J and curve configuration ratio (white) 0.79 were used. LAMBRECHT 80 PALM-H (Tecom Ingredients, Spain), a free-flowing, creamy-white, spray-dried powder containing 80% hydrogenated palm oil, maltodextrine, caseinates and a free-flowing agent as vegetable fat and LAKTEIN 30 (Lacto serum France, France) a free lactose and demineralized lactoserum were added. Commercial compressed yeast (CCY) (10^{10} cells/g, dry matter) was used as a starter. Enzymes included NOVAMYL 10000 BG, maltogenic bacterial α-amylase in granulate form (NMYL), PENTOPAN MONO BG, 1,4-endoxylanase in granulate form (PTP), both from Novozymes (Denmark), and MICROBIAL TRANSGLUTAMINASE ACTIVA WM, glutaminyl-peptide-γ-glutamyl transferase in fine powder form (TGM) from Apliena (Spain).

Dough and bread preparation

The qualitative basic dough formula consisted of fermented sponge, flour, water, salt, lactoserum, sucrose, vegetable fat, calcium propionate and glacial acetic acid (Table 1). Enzymes were added according to the experimental design (Table 2). Process variables (qualitative and quantitative independent factors) tested at two levels (0, 1) included flour (white, whole), NMYL (0, 7.5 mg/100 g flour), PTP (0, 30 mg/100 g flour), and TGM (0, 500 mg/100 g flour). Sponge (sponge dough process) was prepared by mixing the ingredients flour (100 g white/whole), water (60.6/66.0 mL, white/whole) and CCY (14.28 g) in an arm mixer for 14/10 min and fermenting for 2 h at 28 °C, overnight at 5 °C and 1.5 h at 28 °C before addition into bread doughs. Unfermented bread doughs were prepared by mixing the ingredients (basic, additives and enzymes) in a 10-kg mixer at 60 rpm up to optimum dough development. Fermented doughs were obtained after bulk-fermentation (30 min), dividing (600 g doughs), rounding, resting (10 min), panning and proofing up to maximum volume increment (1 h). Fermented doughs were baked at 190 °C for 20 min to make pan breads. After 2 h of cooling, fresh breads were packaged in coextruded polypropylene bags for up to 20 days of storage at 22 °C.

Table 1 Dough formulation of pan breads.

Ingredients	Sponge	Dough
Flour (g)	50	50
Water (mL) (white flour/whole flour)	30.3/33	30.3/33
Compressed yeast (g)	7.14	0
Salt (g)		2.4
Lactoserum (g)		4.8
Sucrose (g)		7.14
Hydrogenated vegetable fat (g)		4
Calcium propionate (g)		0.3
Glacial acetic acid (mL)		0.05
Fermented sponge (g) (white flour/whole flour) (2 h at 28 °C, overnight at 5 °C, 1.5 h at 28 °C)		87.44/90.14

Table 2 Factorial design 2^4 for sampling.

Sample no.	Code	Factors[a]			
		A	B	C	D
1	B-1	0	0	0	0
2	B-2	0	1	0	0
3	B-3	0	0	1	0
4	B-4	0	0	0	1
5	B-5	0	1	1	0
6	B-6	0	0	1	1
7	B-7	0	1	0	1
8	B-8	0	1	1	1
9	I-1	1	0	0	0
10	I-2	1	1	0	0
11	I-3	1	0	1	0
12	I-4	1	0	0	1
13	I-5	1	1	1	0
14	I-6	1	0	1	1
15	I-7	1	1	0	1
16	I-8	1	1	1	1

[a] Levels (0, 1) of factors (A–D). A is flour: white (0), whole (1). B is NOVAMYL 10000 BG, maltogenic bacterial α-amylase in granulate form (*NMYL*): none (0), 0.0075% flour basis (1). C is PENTOPAN MONO BG, 1,4-endoxylanase in granulate form (*PTP*): none (0), 0.030% flour basis (1). D is MICROBIAL TRANSGLUTAMINASE ACTIVA WM, glutaminyl-peptide-γ-glutamyl transferase in fine powder form (*TGM*): none (0), 0.5% flour basis (1).

Static textural measurements

Primary and secondary mechanical characteristics (texture profile analysis in a double compression cycle) were recorded with a TA-XT2i texturometer (Stable Micro System, Surrey, UK) using a 5-cm diameter stainless steel probe, 25-kg load cell, 50% penetration depth and a 30-s gap between compressions, on 20-mm-width slices.

Dynamic viscoelastic measurements

A DMA 7e instrument (PerkinElmer, Norwalk) with a 10-mm parallel-plate geometry was employed for mechanical and thermal analysis. The storage modulus (E'), the loss modulus (E''), and tanδ (E''/E') were recorded and plotted as functions of frequency or temperature (Pyris Software for Windows, PerkinElmer). Samples were submitted to a sinusoidal deformation in order to evaluate bread crumb viscoelastic properties. All bread loaves were uniformly sliced to a thickness of 20 mm. Cylindrical crumb samples (10-mm diameter) were cut from the centres of each bread loaf using a circular cutter and silicon paste (Panreac Quimica) was used to protect the samples from dehydration during the test.

DMA measurement

Cylindrical bread crumb aliquots were placed between the parallel plates at room temperature (25 °C) and then a static force of 15 Nm and a dynamic force (sinusoidal) of 12 Nm were applied in the compression mode during the frequency sweep (1–40 Hz). The forces were small enough to stay within the linear viscoelastic region. The configuration of the instrument required the static force to be about 20% greater than the dynamic force to keep the probe in continuous contact with the sample.

TMA measurement

Cylindrical bread aliquots were placed between the parallel plates, frozen to –40 °C with liquid N_2, and heated from –40 to 80 °C at a rate of 5 °C/min. Stress was applied in the compression mode at a frequency of 5 Hz. Static and dynamic force control options were selected in order to keep the sample inside the range of the linear viscoelastic region during the test. To determine the phase transition temperature (T_g), the tanδ curve was analysed using Pyris Software, PerkinElmer.

Sensory analysis

Sensory analysis of fresh and stored breads [23] was performed with a panel of trained judges using semistructured scales, scored 1–10, in which extremes were described [24]. Evaluated attributes were grouped into visual (cell uniformity, size, brightness and shape, and cell wall thickness), textural (tactile moistness, elasticity and smoothness, and biting coarseness, adhesiveness, cohesiveness, chewiness, crumbliness and dryness) and organoleptic (taste intensity, quality, saltiness, sourness and aftertaste, and aroma intensity, quality and sourness) characteristics.

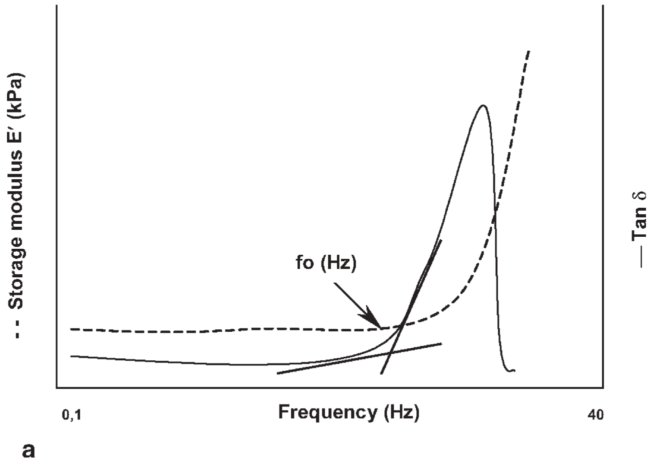
Statistical analysis

Sampling was conducted according to the factorial design structure 2^4 shown in Table 2. Multivariate analysis (correlation matrix and multiple analysis of variance) was performed by using the Statgraphics V.7 program (Bitstream, Cambridge, MA).

Results and discussion

Texture determinations of stored pan breads supplemented with enzymes

Fundamental rheological data from dynamic and thermal mechanical measurements provide simultaneous elastic and viscous responses of the material during storage (Fig. 1). The transition from rubberlike to glasslike consistency can be obtained by evaluating the onset frequency (f_0) [20], considering the frequency dependence of viscoelastic properties, or by determining the glass-transition temperature (T_g) as a function of temperature [17]. f_0 and T_g are the most important dynamic parameters derived from DMA and TMA measurements, respectively. In addition, the E' values at f_0, at T_g, and in the rubbery plateau (E'_p), have been proved to significantly change with bread staling. Most of the quality characteristics of food can be preserved when it is stored at a temperature below its characteristic T_g. Several ingredients may affect T_g and in recent years the significance of T_g has been recognized as a physicochemical event that affects the product quality and stability [19].



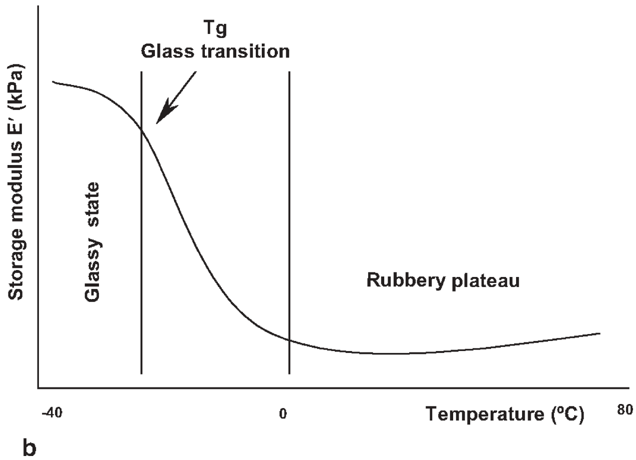


Fig. 1 **a** Typical dynamic mechanical analysis curve in the wide frequency sweep. **b** Typical dynamic thermomechanical analysis curve in the wide frequency sweep.

Statistical design (Table 2) allowed the identification of all single effects (Table 3) and second-order interactions (Table 4) of the design factors (NMYL, PTP, TGM and storage time) on the dynamic and static textural parameters. In order to determine properly these effects, whole and white breads were evaluated separately as the type of flour would mask the effect of the remaining design factors.

Storage time significantly (P<0.01) modified most properties which may, therefore, be used as indicators of bread staling. There was an increase, during storage, in f_0 and $E'(f_0)$ up to 90 and 450%, respectively, in agreement with previous findings [20]. $E'(T_g)$ rose as well (215%), although T_g did not vary with time, probably because there was no change in the moisture content of the bread [5, 17]. However, some ingredients and/or antistaling agents may affect the T_g values [25]. In this work, it was found that TGM (in both flours) and PTP in white flour increased T_g (3.5%), which means less flexibility of the material [25]. This could be explained by the dough-strengthening effect of the cross-linking enzyme [8, 9] and the counteracted gluten aggregation induced by xylanase [26]. On the other hand, NMYL decreased T_g (4.3%) in whole breads in accordance with its antistaling effect.

As widely reported in the literature, storage time significantly influences static texture properties assessed with texturometer readings [11]. Hardness, firmness (sensory evaluation) and chewiness increased with time; conversely, springiness, cohesiveness and resilience, which should be kept as high as possible, significantly decreased (Table 3).

Suitable significant single effects of NMYL were found on most dynamic and static evaluated properties except for f_0, T_g and springiness in white samples and f_0 and $E'(f_0)$ in whole breads. In contrast, the behaviour of breads formulated with PTP or TGM corresponded with those of poor quality. It has to be pointed out that TGM significantly influenced T_g only in whole breads; whereas in white samples, prominent influences on T_g and on most of the static mechanical properties were found. As a general consideration, all effects were higher in white breads (Table 3).

Several second-order interactions regarding enzyme/enzyme (Table 4) and enzyme/time have been found for both white and whole breads. Addition of NMYL to PTP-containing samples significantly improved E'_p in white breads and E'_p, chewiness, resilience and firmness in whole breads. The NMYL decreasing effect on $E'(T_g)$ was even greater if TGM was also present (up to 40%). The PTP/TGM pair combined synergistically leading to a decrease in E'_p and an increase in T_g (41 and 7%, respectively) in white samples to a much greater extent than expected from additive individual actions. The enhancement of f_0 induced by PTP in whole breads was decreased when TGM was in the mixture. The hardness increases with time of storage, but addition of NMYL made this evolution slower for breads containing NMYL stored for the same time were significantly softer. The same trend was found for cohesiveness as NMYL made milder the increases of this parameter. In contrast, PTP promoted an increase in hardness with time.

Table 3 Single effects of design factors on dynamic and thermal properties of stored white and whole pan breads formulated with enzymes. See Table 2 for levels of design factors. The *single asterisk* is P<0.05; the *double asterisk* is P<0.01

Flour	Property	Units	Grand mean	NMYL			PTP			TGM			Time				
				0	1		0	1		0	1		0	1	2	3	
White	f_0	Hz	12.10	13.03	11.17		12.08	12.11		12.24	11.95		8.23^{a}	11.18^{b}	13.30^{c}	15.68^{d}	**
	$E'(f_0)$	kPa	117	152	81	**	118	115		129	104		39^{a}	89^{b}	124^{c}	214^{d}	**
	T_g	ºC	-28.33	-28.39	-28.26		-28.86	-27.80	**	-28.84	-27.82	**	−28.52	−28.33	−28.11	−28.36	
	$E'(T_g)$	kPa	3,496	4,021	2,970		4,173	2,818	*	4,060	2,932		1,971^{a}	1,898^{a}	3,900^{b}	6,212^{c}	**
	E'_p	kPa	183	230	137	*	208	159		210	156		114	155	201	264	
	Hardness	g	1,583	1,924	1,242	**	1,556	1,610		1,577	1,588		728^{a}	1,403^{b}	1,726^{c}	2,474^{d}	**
	Springiness		0.970	0.969	0.971		0.974	0,967	**	0.969	0.971		0.985^{a}	0.975^{b}	0.964^{c}	0.956^{d}	**
	Cohesiveness		0.594	0.542	0.645	**	0.605	0.582		0.593	0.595		0.724^{a}	0.614^{b}	0.537^{c}	0.501^{d}	**
	Chewiness		839	927	750	**	844	834		842	836		517^{a}	827^{b}	879^{b}	1132^{c}	**
	Resilience		0.236	0.206	0.265	**	0.245	0.226	*	0.231	0.241		0.333^{a}	0.238^{b}	0.195^{c}	0.176^{d}	**
	Firmness	1–10	2.56	3.25	1.88	**	2.31	2.81		2.71	2.42		1.38^{a}	1.88^{a}	2.75^{b}	4.25^{c}	**
Whole	f_0	Hz	13.27	13.03	13.52		12.81	13.73	*	12.96	13.59		10.89^{a}	12.33^{b}	13.26^{c}	16.62^{d}	**
	$E'(f_0)$	kPa	126	125	128		104	149	**	120	132		84^{a}	108^{a}	137^{b}	183^{c}	**
	T_g	°C	−28.19	−27.59	−28.79	*	−28.26	−28.12		−28.71	−27.67	*	−28.26	−28.07	−28.15	−28.28	
	$E'(T_g)$	kPa	3,130	3,795	2,465	**	2,960	3,300		3,185	3,075		2,058^{a}	2,709^{b}	3095^{c}	4658^{d}	**
	E'_p	kPa	362	431	292	*	377	346		358	365		285	322	355	485	
	Hardness	g	2,337	2,684	1,989	**	1,991	2,682	**	2,032	2,642	**	1,343^{a}	2,072^{b}	2580^{c}	3352^{d}	**
	Springiness		0.938	0.933	0.943	*	0.941	0.936		0.938	0.939		0.958^{a}	0.940^{b}	0.932^{c}	0.923^{d}	**
	Cohesiveness		0.510	0.465	0.555	**	0.517	0.503	*	0.517	0.503	*	0.614^{a}	0.512^{b}	0.470^{c}	0.444^{d}	**
	Chewiness		1,055	1,100	1,010	*	920	1,189	**	926	1,183	**	787^{a}	983^{b}	1,112^{c}	1,338^{d}	**
	Resilience		0.176	0.152	0.200	**	0.185	0.167	**	0.178	0.173		0.238^{a}	0.172^{b}	0.152^{c}	0.142^{c}	**
	Firmness	1–10	3.09	4.36	1.81	**	2.67	3.50	*	2.69	3.49	*	1.75^{a}	2.63^{b}	3.63^{c}	4.35^{c}	**

Table 4 Second-order interactive effects of design factors on dynamic and thermal properties of stored white and whole pan breads formulated with enzymes. See Table 2 for levels of design factors. The *single asterisk* is $P<0.05$; the *double asterisk* is $P<0.01$

Property	Units	Level	NMYL/PTP		NMYL/TGM		PTP/TGM	
			White	Whole	White	Whole	White	Whole
$E'(f_0)$	kPa	00						87^{a*}
		01						121^{b}
		10						154^{c}
		11						144^{cb}
		Mean						126
T_g	°C	00		-27.28^{a**} -28.88^{a**}	-29.09^{a*}			
		01		-27.90^{a}	-27.70^{b}		-28.84^{a}	
		10		-30.14^{b}	-28.58^{c}		-28.80^{a}	
		11		-27.44^{a}	-27.94^{b}		-26.80^{b}	
Mean		−28.19	−28.33		28.33			
$E'(T_g)$	kPa	00				$3{,}561^{a**}$		$3{,}372^{a**}$
		01				$4{,}028^{b}$		$2{,}548^{b}$
		10				$2{,}809^{c}$		$2{,}997^{c}$
		11				$2{,}122^{d}$		$3{,}603^{a}$
		Mean				3,130		3,130
E'_p	kPa	00	279^{a*}	517^{a*}			247^{a**}	503^{a**}
		01	180^{b}	345^{b}			167^{b}	251^{b}
		10	135^{c}	237^{b}			172^{b}	213^{b}
		11	138^{c}	348^{b}			145^{b}	480^{a}
		Mean	183	362			183	362

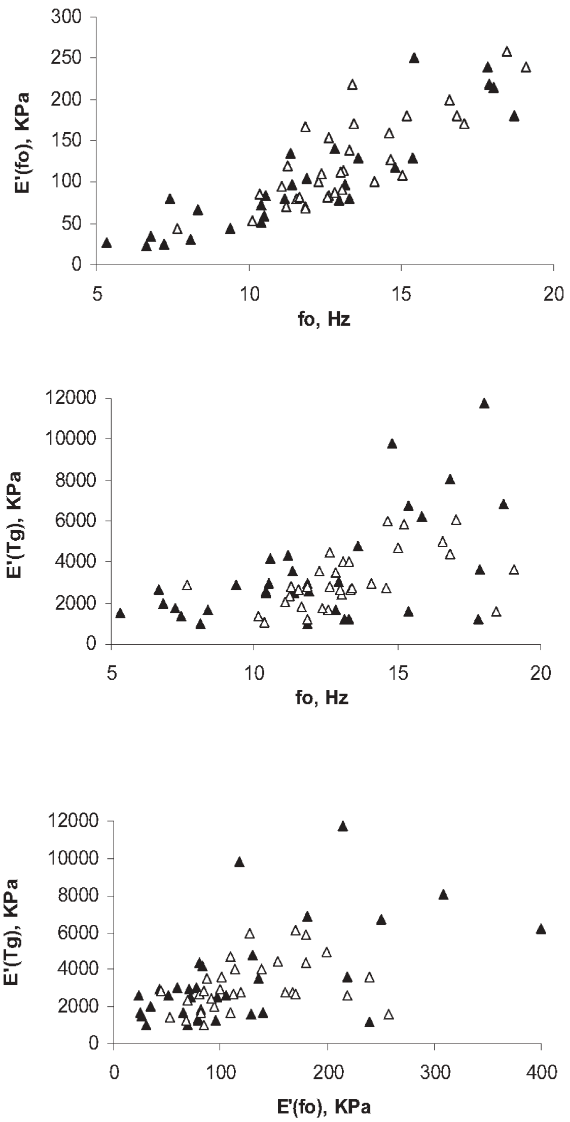


Fig. 2 Correlations within dynamic mechanical and thermal measurements. ▲ white bread △ whole bread

Relationships within dynamic thermomechanical, static and sensory characteristics 6in supplemented pan breads

Multivariate data handling of bread crumb functional variables supplied useful information on the significantly correlated texture properties of formulated fresh and aged samples. Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.46 to 0.83) were obtained for the relationships between dynamic thermomechanical analysis (DTMA), TPA and firmness parameters (Figs. 2, 3, 4). Figure 2 shows that $E'(f_0)$ and $E'(T_g)$ were positively correlated versus f_0 (r=0.83; r=0.47); the results obtained are in accordance with the fact that all those parameters increased in aged breads. A positive correlation (r=0.46) was also found within thermal and mechanical properties, $E'(T_g)$ and $E'(f_0)$. These data support the potential interchangeability between dynamic mechanical and thermal measurements in the evaluation of the crumb structural changes during storage. Other authors [20] reported similar behaviour [rise in $E'(f_0)$ and in f_0 with time], in contrast to that reported in Ref. [17] for $E'(T_g)$, where no changes in the E' values between fresh and aged bread were found. The relationships between dynamic and static measurements (Fig. 3) evidenced negative correlations (r from −0.50 to −0.68) between static parameters (cohesiveness and resilience) considered as bread quality indicators [2] and the viscoelastic dynamic measurements. Positive correlations (r from 0.48 to 0.66) between hardness and f_0, $E'(f_0)$ and E'_p were found as previously stated [20]. Finally, positive correlations (r=0.53; r=0.49) were obtained for firmness (measured by sensory analysis with a panel of trained judges) and $E'(f_0)$

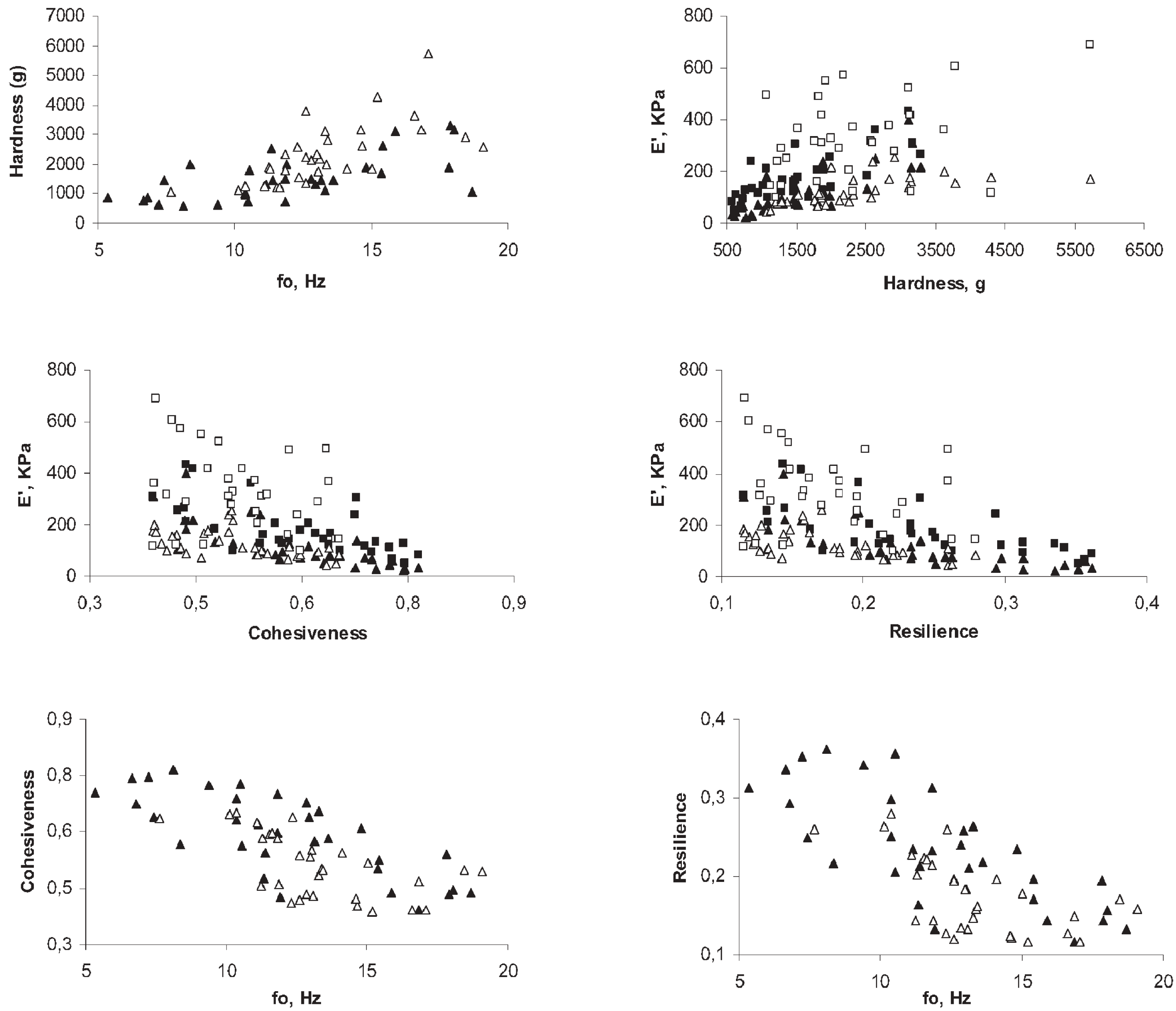
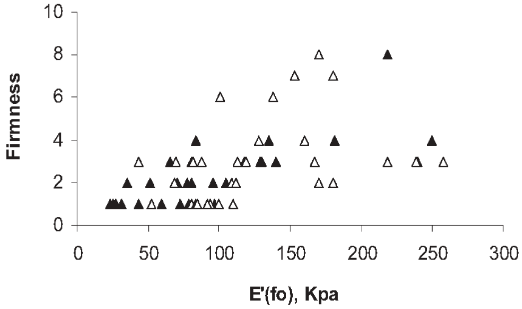


Fig. 3 Correlations within dynamic mechanical and thermal measurements versus static parameters. Whole bread $E'(f_0)$ —storage modulus in the onset frequency (*open triangles*); whole bread $E'(f_p)$—storage modulus in the rubbery plateau (*open squares*); white bread $E'(f_0)$ (*closed triangles*); white bread $E'(f_p)$ (*closed squares*)

and $E'(T_g)$ (Fig. 4). In addition, hardness, chewiness and firmness were positively correlated (r from 0.47 to 0.64) with $E'(T_g)$, $E'(f_0)$ and f_0, respectively. Springiness, cohesiveness and resilience were negatively correlated (r from –0.38 to –0.51) with all the dynamic parameters except for T_g. All these results indicate that the dynamic viscoelastic parameters [$E'(f_0)$, $E'(T_g)$, E'_p and f_0] would have a significant role in monitoring bread staling.

Conclusions

Small dynamic deformation (in fundamental units) and large static deformation methods can be used in order to evaluate the thermodynamical and physicomechanical changes of bread during staling. Rheological changes in bread associated with recipe and storage time were successfully detected via dynamic thermomechanical analysis in the compression mode. Bread crumbs behaved similarly to a soft rubberlike solid below the onset frequency and at a temperature above T_g. Typical viscoelastic behaviours of bread concerned a transition from rubberlike to glasslike consistency or vice versa with increasing frequency or temperature, respectively. The onset frequency (f_0) and the rubbery or plateau moduli (E') rose as the bread aged in a similar way to the hardening and firming curves. Relationships between the dynamic (DTMA) and static (texture analysis) methods were found. All the bread quality indicators measured by static methods were negatively correlated with the viscoelastic dynamic parameters.



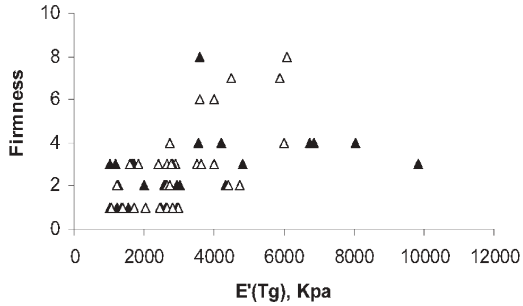


Fig. 4 Correlations within dynamic mechanical and thermal measurements versus firmness (measured by sensory analysis). White bread (*closed symbols*); whole bread (*open symbols*)

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