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Staling Behavior of Chickpea-Enriched Bread: A Dynamic Mechanical Analysis Approach

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ABSTRACT

Bread is a staple food widely consumed worldwide and therefore represents an excellent vehicle for increasing nutrient intake. In this context, the incorporation of chickpea flour into bread offers promising nutritional benefits; however, its impact on bread technological quality remains underexplored. This study investigated staling phenomena in composite breads produced by substituting wheat flour with chickpea flour at 0%, 10%, 20%, and 30%. Moisture content, texture, and viscoelastic properties were monitored over 7 days of storage. Dynamic mechanical analysis (DMA), including frequency sweep and temperature scan tests, was employed as a less commonly used tool to evaluate mechanical changes associated with staling and formulation. Multivariate analyses were used to assess the effects of chickpea flour and storage time on bread properties. Hardness was found to be correlated with viscoelastic parameters obtained by the DMA *frequency sweep* test. The DMA *temperature scan* was able to measure the mechanical changes associated with frozen water transition, showing a significant decrease in ice melting temperature related to chickpea flour inclusion (~3°C) and storage time (~2.5°C). Among the different parameters used to describe thermal transitions (storage and loss moduli drops and tanδ peak), it was identified which of these was most effective in discriminating the samples as an effect of both chickpea flour and storage time. Overall, these results support the potential of DMA as a complementary analytical approach for assessing staling and mechanical changes evolution in chickpea-enriched bread, providing new insights into formulation-induced structural changes during storage.

1 | Introduction

The consumption of bread has reached a very high level all over the world (FAOSTAT 2022). By increasing bread nutritional value, this trend can be harnessed to have a positive impact on human health. Pulses can be introduced in the bread formulations for this purpose, as they contain a higher content of high-quality protein, fat, ash, and other bioactive compounds compared to wheat (Jukanti et al. 2012; Rachwa-Rosiak et al. 2015; Wallace et al. 2016). Bread formulation can be enriched with pulses in a variety of forms, from hulled and dehulled flours, hydrolysates or protein isolates, germinated or fermented pulse flours, and from a single pulse type to mixtures of different pulses (Boukid et al. 2019). Chickpea (*Cicer arietinum* L.) is a legume crop with great potential for the following reasons. From 2000 to 2022, the chickpea global production increased from 8 million tons to 18 million tons, with India leading the production with 13 million tons in 2022 (FAOSTAT 2024). The addition of chickpea flour to bread can improve its nutritional value, particularly by increasing the essential amino acids and dietary fiber intake (Sulieman et al. 2013).

However, numerous studies suggested that the addition of chickpea flour to wheat bread affects its structure (Mohammed et al. 2012; Vinod et al. 2023; Yamsaengsung et al. 2010). Indeed, the presence of non-gluten proteins can hinder the formation of the gluten network, thus resulting in an increase in water absorption and dough development time (Mohammed et al. 2012;

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Mohammed et al. 2014). This can in turn worsen the volume and firmness of the bread loaf, as well as the color and sensory properties. The specific effects vary according to the variety (due to a possible different composition of the seeds) and the amount of chickpea flour used in the formulation (Atudorei et al. 2022; Bresciani et al. 2024; Hallén et al. 2004; Noort et al. 2010; Yamsaengsung et al. 2010). If the inclusion of chickpea flour in bread formulation can be a great opportunity for nutritional purposes, on the other hand, great effort has to be devoted to the study of the effect of the ingredient at the technological level considering the impact on both the quality of fresh bread and during storage.

Bread staling is a complex process that occurs during storage and results in the hardening of the crumb, softening of the crust, and loss of the characteristic fresh bread flavors (Fadda et al. 2014; Gray and Bemiller 2003). This phenomenon is due to several interrelated factors. An important contributor is the recrystallization of starch leading to crumb hardening, together with the dehydration and subsequent loss of plasticity of gluten and changes in the interactions between gluten and starch (Baik and Chinachoti 2001; Amigo et al. 2021; Liu et al. 2024). In this complex picture, water plays a crucial role in bread staling, as its migration from the crumb to the crust and redistribution at the molecular level significantly influences the texture and flavor of bread. During storage, water is incorporated into starch crystals and redistributed to the different bread domains (Slade and Levine 1991; Schiraldi and Fessas 2001; Vittadini and Vodovotz 2003; Curti et al. 2014; Liu et al. 2024).

Dynamic mechanical analysis (DMA) is a valuable tool for studying the viscoelastic properties of bread, providing insight into different thermal transitions and their relations with moisture content (Hallberg and Chinachoti 1992). This technique can also detect changes in bread rheology caused by recipe variations and storage time (Bollaín et al. 2005). DMA demonstrated its usefulness in studying the thermomechanical properties of stored wheat bread in numerous studies, theoretically offering 1000 times greater sensitivity than differential scanning calorimetry (DSC) (Baik and Chinachoti 2000; Bollaín et al. 2005; Hallberg and Chinachoti 1992; Hallberg and Chinachoti 2002; Ribotta and Le Bail 2007; Rotter and Ishida 1992; Wetton 1998).

No previous studies have utilized DMA to investigate the mechanical properties of pulses-composite bread during storage. Thus, this study aims to determine whether differences in the mechanical transitions of bread can be detected as an effect of chickpea flour addition and storage time. This work responds to the demand for the development of high-quality composite breads for nutritional purposes by studying dynamic-mechanical properties of bread during storage.

2 | Materials and Methods

2.1 | Bread Formulation and Production

The ingredients used in bread formulations were wheat flour (Agugiaro&Figna s.p.a., Collecchio, Italy), chickpea flour (MartinoRossi Spa, Gadesco, Italy), salt (Italkali s.p.a., Palermo, Italy), sunflower seeds oil (Oleificio Zucchi, Cremona, Italy), sugar (Italia Zuccheri, Bologna, Italy), and yeast (AB Mauri s.p.a., Casteggio, Italy). Wheat flour composition (as indicated by the producer) was as follows: proteins 11.0%, carbohydrates 71.0% (of which sugars 1.5%), fats 0.9%, and fibers 2.0%. Chickpea flour composition (as indicated by the producer) was as follows: proteins 22.8%, carbohydrates 47.9% (of which sugars 2.8%), fats 5.8%, and fibers 10.8%. Composite bread samples were produced by substituting wheat flour with 0% (STD), 10% (C10), 20% (C20), and 30% (C30) of chickpea flour. Based on the manufacturer's technical data sheets of wheat flour and chickpea flour, the composition of the composite flours was calculated for each substitution level (0%, 10%, 20%, and 30%) and reported in Table 1. The water amount used in bread formulations was determined based on the water necessary to obtain dough samples with a reference consistency of 500 BU (Brabender Farinograph) (AACC 2010) (data not shown) and equal to 55.7, 57.3, 57.7, and 57.2 (g/100 g of flour) for STD, C10, C20, and C30, respectively. STD bread formulation was based on previous works (Del Vecchio et al. 2025; Parenti et al. 2025) and reported in Table 1. Bread production was carried out with a home bread machine (Moulinex Uno, OW3101) to avoid any production variable able to affect bread data variability. The following baking procedure was used: 34 min mixing, 25 min resting (1st leavening, 30°C), 1 min mixing, 25 min resting (2nd leavening, 35°C), 1 min mixing, 45 min resting (3rd leavening, 35°C), and 58 min baking (150°C) (Gigliotti et al. 2025). Then, bread loaves were cooled at room temperature for 2 h before being placed in sealed polyethylene bags. Four milliliters of ethanol was sprinkled into the bags, and samples were stored at 25°C for 7 days. Bread loaves were analyzed fresh (Day 0) and after 2, 5, and 7 days from production. For each production batch ($n = 2$), two bread loaves were obtained and analyzed at each storage time.

TABLE 1 | Composition of composite flour blends: all components were calculated from manufacture's technical sheets and expressed as g/100 g of composite flour. Bread formulation: flours ingredients are expressed as g; water, sugar, yeast, sunflower oil, and salt are expressed as g/100 g of flour.

		STD	C10	C20	C30
Composite flours composition	Fat	0.9	1.4	1.9	2.4
	Carbohydrate	71	68.7	66.4	64.1
	Sugar	1.5	1.6	1.8	1.9
	Protein	10	11.3	12.6	13.8
	Fiber	2	2.9	3.8	4.6
Bread formulations	Wheat flour	100	90	80	70
	Chickpea flour	0	10	20	30
	Water	55.7	57.3	57.7	57.2
	Sugar	4	4	4	4
	Yeast	3	3	3	3
	Sunflower oil	3	3	3	3
	Salt	2	2	2	2

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2.2 | Moisture Content

Moisture content (MC, g of water/100 g of sample) of bread crumb and crust was measured by analyzing the sample weight loss after drying in a forced-air oven (NSV 9035, ISCO, Milan, Italy) at 105°C until constant weight. Three measurements were performed for each loaf.

2.3 | Texture

Bread crumb texture was measured using a Texture Analyzer TA.TX2 (Stable Micro Systems, Godalming, UK). A texture profile analysis (TPA) test was performed with a cylindrical probe (P/35 Dia Cylinder Aluminum) on at least six cubic portions ($2 \times 2 \times 2\,cm^3$) of crumb extracted from the central slices of bread loaves. The crumb cubes were compressed to 40% deformation with a speed of 1 mm/s and a trigger load of 0.05 N. From the force-deformation curve, hardness (N) was calculated as the maximum load on Cycle 1, springiness (-) as the length of Cycle 2 (during the second compression) divided by the length of Cycle 1 (during the first compression), and cohesiveness (-) was determined as the total positive area in Cycle 2 divided by the total positive area in Cycle 1.

2.4 | Dynamical Mechanical Properties

Dynamic mechanical properties were measured using a Q800 Dynamic Mechanical Analyzer (TA Instruments, New Castle, DE, USA). Two different tests were performed. The first test was a *temperature scan*, and the DMA was equipped with the single-cantilever geometry. A bread crumb-pressed sample with a rectangular shape ($50 \times 12 \times 2\,mm^3$) was used for each measurement. Dynamic storage modulus (E′) and loss modulus (E″) were monitored from −50°C to 120°C with a heating rate of 3°C/min at a controlled sinusoidal strain (maximum strain 0.1%) and with a constant frequency (1 Hz). The ratio between E″ and E′ was calculated to obtain the damping factor, tanδ. By processing the data of E′, E″, and tanδ curves (Universal Analysis software, version 3.9A, TA Instruments, DE, USA), different parameters were obtained. For clarity of interpretation, Figure S1 illustrates the parameters selected to describe the thermal transitions and the corresponding signals. From the storage modulus signal, the following parameters were measured (Figure S1a): the temperature at which the drop of E′ started (T-E′ [°C]), the temperature range in which E′ dropped (ΔT-E′ [°C]), the values of E′ modulus before and after the drop (E′-before [MPa] and E′-after [MPa], respectively), and the change in modulus at the drop as measured by the difference between modulus before and after the drop (Δ-E′ [MPa]). From the loss modulus signal (Figure S1b), the following parameters were taken: the temperature at which the modulus started to increase prior to the drop (T-peak-E″ [°C]), the temperature range in which E″ dropped (ΔT-E″ [°C]), the values of E″ modulus before and after the drop (E″-before [MPa] and E″-after [MPa], respectively), and the change in modulus at the drop as measured by the difference between modulus before and after the drop (Δ-E″ [MPa]). Lastly, from tanδ peak signal (Figure S1c), the temperatures corresponding to each detected peak ($n = 3$) were measured (T-1°-tanδ [°C], T-2°-tanδ [°C], and T-3°-tanδ [°C]).

The second DMA test performed was a *frequency sweep* test performed under isothermal condition (25°C), for which the DMA was equipped with a compression clamp using parallel plate (15-mm diameter). Crumb cylinders (2-mm thickness and 10-mm diameter) were sampled from the center of each bread loaf. A frequency range of 1–40 Hz was applied. Amplitude was set to 10 μm, a value within the linear viscoelastic region, as preliminary determined by a *strain sweep* test (amplitude 0.1–100 μm; frequency 1 Hz; temperature 25°C) (data not shown). E′, E″, and tanδ evolutions as a function of frequency changes were measured. The curves obtained were processed using TA Universal Analysis software (version 3.9A, TA Instruments, DE, USA), to obtain E′, E″, and tanδ values at 1 and 10 Hz (E′-1 Hz [MPa]; E′-10 Hz [MPa]; E″-1 Hz [MPa]; E″-10 Hz [MPa]; tanδ-1 Hz; tanδ-10 Hz) (Figure S2).

For both DMA tests, at least five replicates for each batch of bread were performed.

2.5 | Statistical Analysis

Firstly, a principal component analysis (PCA) was carried out to obtain an overview of all the collected physico-chemical parameters of bread (*x* variables), using SIMCA17.0.1 Software (Version 17.0.1, Umetrics Suite, Sartorius Stedim Biotech, Umeå, Sweden). The PCA *x* variables were obtained from the four types of bread (STD, C10, C20, and C30) at four different storage times (0, 2, 5, and 7 days), resulting in a total of 16 observations: STD_0, STD_2, STD_5, and STD_7; C10_0, C10_2, C10_5, and C10_7; C20_0, C20_2, C20_5, and C20_7; C30_0, C30_2, C30_5, and C30_7. All the data were preprocessed by mean-centering and unit variance scaling (UV–scaling) before creating the PCA model. After getting an overview of the dataset with the PCA model, two "Orthogonal – Partial Least Squares" (OPLS) models were created, in order to analyze the relationship between the measured parameters and the two independent variables considered: chickpea flour content (%) (Model 1) and storage time (days) (Model 2). Response variables with a variable importance in projection (VIP) lower than 0.8 were excluded from the model since less affected by independent variables (Akarachantachote et al. 2014; Chong and Jun 2005).

Lastly, a one-way analysis of variance (ANOVA) (SPSS software 27.0, SPSS Inc., Chicago, USA) was used to verify the presence of significant differences between the means of the response variables resulted mostly affected by the independent variables chickpea flour content and storage time. In the case of homoscedastic datasets and homogeneous sample sizes, a Tukey post hoc test with a confidence interval of 95% was used, while in the case of different sample sizes, a post hoc least significant difference (LSD) test was used, with the same confidence interval. Instead, in the case of heteroscedasticity, the Welch test followed by Games–Howell post hoc test with a 95% confidence interval was used. Correlation coefficients (*r*) were computed using Pearson's coefficient test to assess the linear relationship between select variables ($p \leq 0.05$).

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3 | Results

3.1 | Qualitative Analysis of DMA Outputs

The most commonly used DMA tests in bread analysis are the *frequency sweep* and the *temperature scan* tests. These tests apply a sinusoidal stress to measure deformation, allowing the measurement of complex (E*), storage (E′), and loss (E″) moduli as key indicators of the mechanical properties of bread (Menard and Menard 2020).

DMA curves resulting from the *frequency sweep* test are shown in Figure S3. In the frequency range explored, all samples showed a storage modulus (E′) greater than the corresponding loss modulus (E′), and a tanδ < 1, thus indicating solid-like behavior in all bread samples. As expected, both E′ and E″ moduli showed a general dependence on frequency, with moduli-increasing trend as frequency increased. The magnitude of E′ and E″ moduli also varied following chickpea flour inclusion; in particular, C30 samples exhibited higher E′ and E″ values compared with the other bread formulations. Moreover, in all samples, tanδ increased with increasing frequency until a maximum value was reached (around 35 Hz), and then decreased. The decreasing trend after the peak indicated that the loss modulus increased more than the storage modulus at high frequencies, suggesting that the system changed from a rubbery-like to a glassy-like consistency (Hallberg and Chinachoti 1992; Wang and Sun 2002; Wu et al. 2012).

During a DMA *temperature scan*, a main transition, from a rigid to a more flexible state, can be observed in the mechanical properties of bread samples around 0°C. During the transition, the storage (E′) and loss (E″) moduli decrease significantly. The loss modulus (E″) is often preceded by a small prepeak resulting from the rearrangement of the structure prior to its drop. Furthermore, a peak of tanδ (E″/E′), resulting from changes in the moduli ratio, is also associated with the transition. Figure 1 shows the E′, E″, and tanδ profiles for different bread samples at Day 0, 2, 5, and 7, obtained by the *temperature scan*. All bread samples, at any storage time, exhibited the main transition around ~0°C. According to Vittadini et al. (1996) and Vodovotz et al. (1996), this transition may be relatable to the ice melting as an effect of the temperature increase. The same authors suggested that the ice melting transition in bread appears to dominate thermomechanical events at moisture contents higher than 30%, when freezable water is present (in this study, the moisture content of bread crumb was ~40%, g water/100-g sample). In all samples, another transition was identified at ~80°C; this transition was previously associated with the occurrence of both the melting of amylopectin crystals formed during storage (Baik and Chinachoti 2000; Vittadini et al. 1996; Vodovotz et al. 1996) and water evaporation during sample heating at these temperatures (Baik and Chinachoti 2000). Due to uncertainty regarding its origin, this transition was therefore not further discussed to avoid misinterpretation of the data.

3.2 | PCA

All data (mean ± standard deviation) of the response variables obtained from the different analyses performed are reported in Tables S1 and S2.

A preliminary PCA was conducted with all the response variables to find possible outliers and to provide general

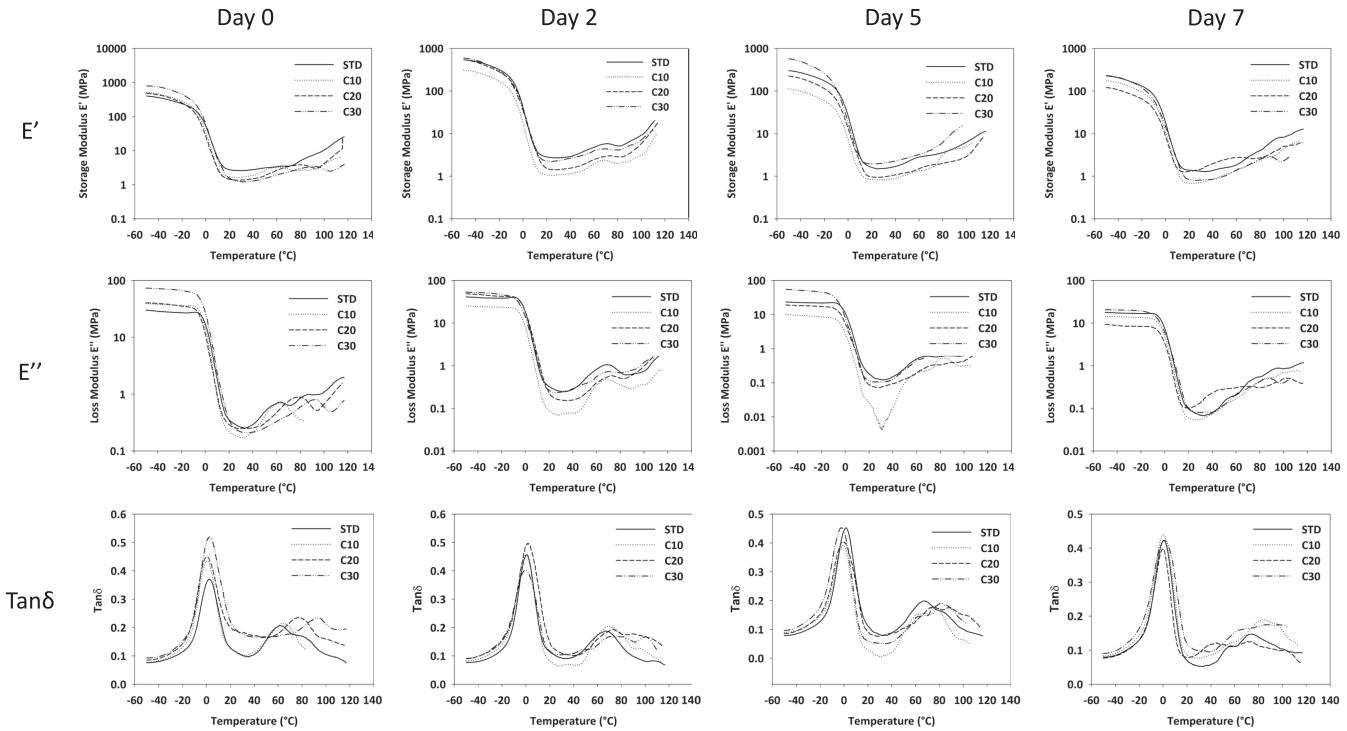

FIGURE 1 | Representative storage modulus (E′), loss modulus (E″), and tanδ profiles for different bread samples at Day 0, 2, 5, and 7, obtained through the temperature scan test.

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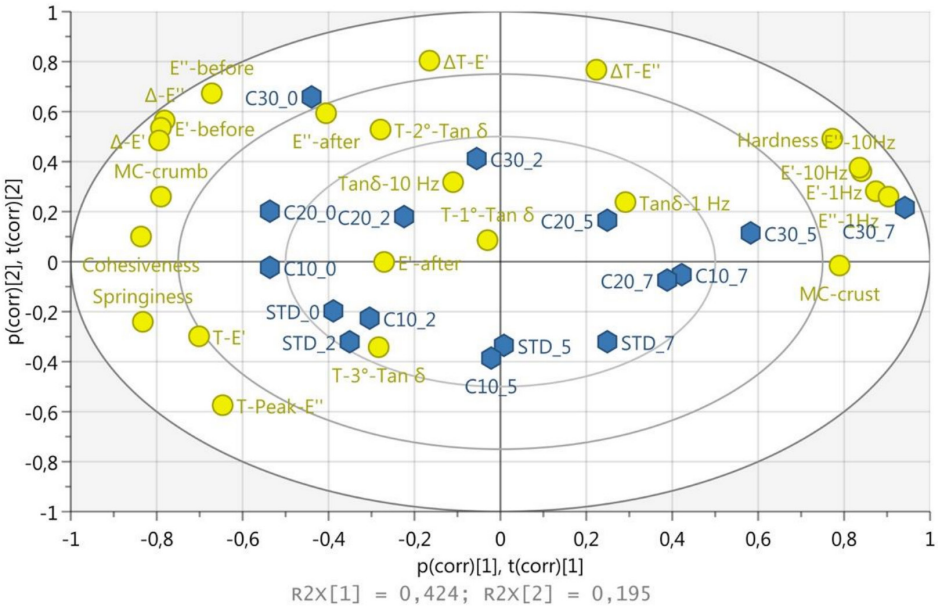

FIGURE 2 | Biplot of the first two components (PCs) of the PCA model; the response variables (yellow) were obtained from the four types of bread (STD, C10, C20, and C30) at four different storage times (0, 2, 5, and 7 days), with a total of 16 samples (blue): STD_0, STD_2, STD_5, and STD_7; C10_0, C10_2, C10_5, and C10_7; C20_0, C20_2, C20_5, and C20_7; C30_0, C30_2, C30_5, and C30_7.

understanding about the relationships between variables (Figure 2). A PCA model with five components was obtained, explaining 75% of the total variance (R^2), with a prediction power of 37% (Q^2). The first two components, PC1 (p(corr)[1]) and PC2 (p(corr)[2]), explained 42.4% and 19.5% of the variance, respectively, and are discussed below. Looking at the scores distribution, PC1 and PC2 appeared to discriminate samples according to storage time and chickpea flour content, respectively. Bread samples at Days 0 and 2 were well positioned in the left quadrants, while bread samples at longer storage time (Days 5 and 7) were located in the right quadrants. Interestingly, the distribution of bread samples as a function of storage time showed that the scores for STD, C10, and C20 were closely positioned, whereas C30 exhibited a wider distribution along PC1, suggesting greater changes during storage compared with the other breads. Along PC2, the spatial distribution of samples was influenced by chickpea flour content, with STD and C10 in the bottom quadrants while C20 and C30 in the upper ones.

Looking at the loadings distribution, the response variables of the DMA *frequency sweep* test (E'-1 Hz, E'-10 Hz, E'-1 Hz, and E'-10 Hz), together with hardness and MC crust, were located in the upper right quadrant, thus presenting high relative values for samples at Days 5 and 7, particularly for C30 bread. In contrast, the same response variables showed lower relative values for samples at Day 0 (distributed on the left quadrant). The loadings that were mostly influencing PC2, and thus most effective in discriminating samples with different chickpea flour contents, were parameters obtained from the DMA *temperature scan*. High values of T-Peak-E'' and T-E' were associated with STD and C10 samples. On the other hand, high values of ΔT-E', ΔT-E'', E'-before, Δ-E', and Δ-E'' were associated with C20 and C30 samples.

3.3 | Orthogonal Partial Least Squares (OPLS)

Two OPLS models were generated considering the relationship between the measured parameters and chickpea flour content (Model 1) or storage time (Model 2). Figure 3a shows the biplot of the OPLS model 1, while the loading plot is reported in Figure S4a. Variables with VIP < 0.8 were excluded from the Model 1 and were as follows: MC-crumb, MC-crust, cohesiveness, tanδ-1 Hz, tanδ-10 Hz, E'-before, E'-after, Δ-E', E''-before, E''-after, Δ-E'', T-1°-tanδ, T-2°-tanδ, and T-3°-tanδ. The resulting model was characterized by $R^2 = 91\%$ and $Q^2 = 83\%$, indicating that the model generated was suitable for describing the relationships between chickpea flour addition and resulting instrumental parameters (and thus the overall bread properties). In the OPLS method, the first component is predictive and describes the direct relationship between *x* and *y* variables, while the second component represents the structured variation in *x* not related to *y*. Therefore, in our OPLS model 1, component 1 (P_1) describes the relationship between the bread measured parameters (*x* variables) and the chickpea flour content (*y* variable), while the orthogonal component (P_2) describes the variation in the bread properties not related to chickpea flour addition in bread formulation. As expected, the samples were distributed along P_1 according to their chickpea flour content, ranging from the lowest (left quadrants) to the highest (right quadrants). The STD and C10 samples were close to each other, whereas C20 was distributed in an intermediate position between C10 and C30, with the latter not clearly distinguishable from C20. About the response variables mostly affected by chickpea flour content and thus accountable for the samples grouping, T-Peak-E'', T-E', and springiness were found to be negatively related with chickpea flour content, assuming negative value along P_1. Indeed, T-Peak-E'', T-E', and springiness presented high relative values for STD and C10,

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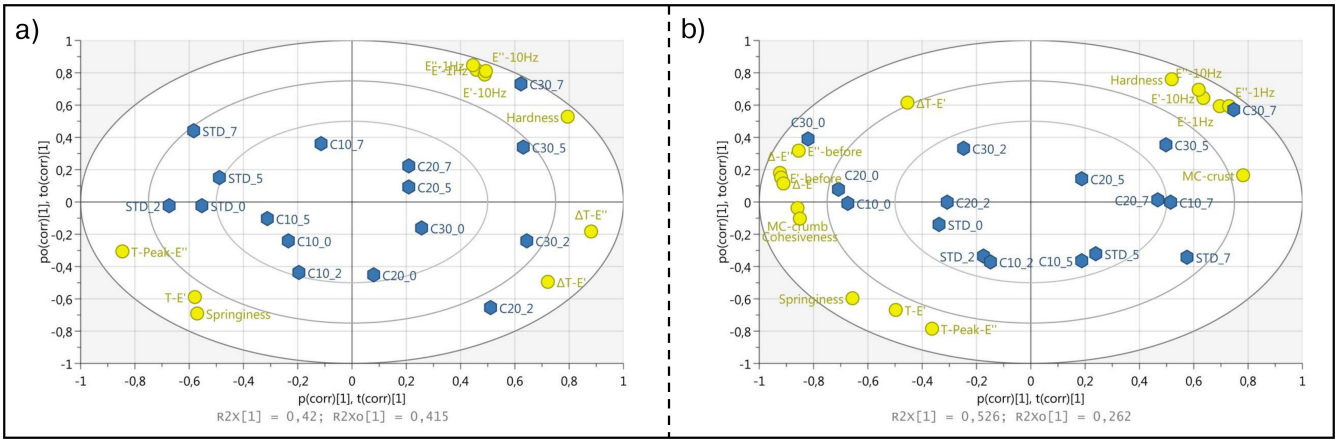

FIGURE 3 | (a) OPLS biplot of Model 1, showing the relationship between bread properties and chickpea flour content. (b) OPLS biplot of Model 2, showing the relationship between bread properties and storage time. The response variables (yellow) were obtained from the four types of bread (STD, C10, C20, and C30) at four different storage times (0, 2, 5, and 7 days), resulting in a total of 16 samples (blue): STD_0, STD_2, STD_5, and STD_7; C10_0, C10_2, C10_5, and C10_7; C20_0, C20_2, C20_5, and C20_7; C30_0, C30_2, C30_5, and C30_7.

confirming the results of PCA. High content of chickpea flour (right quadrants) was positively related with hardness, and to parameters given by the DMA *frequency sweep* test (E′-1 Hz, E′-10 Hz, E″-1 Hz, and E″-10 Hz) and *temperature scan* (ΔT-E′ and ΔT-E″). Indeed, C30 samples were described by high values of these parameters. This insight provided additional information than the considerations made with PCA, where variables ΔT-E′ and ΔT-E″ were positioned in the opposite quadrant to hardness and *frequency sweep* variables. Indeed, the OPLS method highlighted the relationship between bread properties and chickpea flour content, separating the contribution to variation given by other factors (e.g., storage time) that may have had a greater impact than chickpea flour on the overall spatial distribution of samples shown by PCA.

Figure 3b shows the biplot of OPLS model 2, which shows the relationship between the bread measured parameters (*x* variables) and the storage time (*y* variable), while the loading plot is reported in Figure S4b. Also, in this case, variables with VIP < 0.8 were excluded from Model 2: tanδ-1 Hz, tanδ-10 Hz, ΔT-E″, E′-after, E′-before, Δ-E″, T-1°-tanδ, T-2°-tanδ, and T-3°-tanδ. As for Model 1, with an $R^2 = 95\%$ and $Q^2 = 90\%$, the model generated was suitable for describing the effect of storage time on bread instrumental parameters. In this model, the orthogonal component described the variation in the bread properties not related to storage time. Samples of Day 0 and Day 2 were prominently located in the left quadrants of the plot, while samples of Day 5 and Day 7 were positioned in the right quadrants. Notably, sample C30_7 exhibited marked displacement from the other samples of Day 7, indicating a unique behavior. Similarly, sample C30_5, despite representing Day 5 of storage time, positioned close to the other samples at Day 7, suggesting different staling dynamics in different samples. The specific parameters contributing to these differences revealed that samples of Day 5 and, particularly of Day 7, were characterized by high values of hardness, DMA *frequency sweep* test parameters (E′-1 Hz, E′-10 Hz, E″-1 Hz, and E′-10 Hz), and MC crust. Conversely, samples of Day 0 and Day 2 exhibited relatively high values in parameters such as MC crumb, cohesiveness, springiness, and DMA *temperature scan* parameters such as T-E′, ΔT-E′, E′-before, E″-before, E″-after, Δ-E′, and Δ-E″.

4 | Discussion

4.1 | The Effect of Chickpea Flour Content

Based on OPLS model 1 outputs, a one-way ANOVA was carried out for mostly significative response variables, in order to discuss the effect of chickpea flour content on physicochemical properties of bread. Hardness was one of the properties mostly affected by chickpea flour content (Figure 4a). Increasing chickpea flour content in bread formulation significantly increased hardness at each chickpea flour level. Similar results were also found by other authors (Atudorei et al. 2022; Guardado-Félix et al. 2020; Hallén et al. 2004; Ouazib et al. 2016; Xiao et al. 2016; Yamsaengsung et al. 2010), which related the increased hardness to the higher fiber and protein content in chickpea flour than in wheat flour (our composite flours estimated composition is reported in Table 1). At increasing replacement levels, this compositional change may impair gluten network development and gas-cell stabilization, leading to reduced loaf expansion and denser crumb, which in turn contributes to higher firmness. Indeed, Yamsaengsung et al. (2010) reported that chickpea flour incorporation resulted in lower volume and a more compact crumb structure, coherently supporting crumb densification as a plausible contributor to the increase in firmness. Moreover, the increase of insoluble fiber content in bread formulation due to the presence of chickpea flour was reported to interfere with the gluten network formation, reducing the optimal bread crumb viscoelasticity (Matsushita et al. 2019). On the other hand, springiness (Figure 4a) was not affected by the chickpea flour until the 20% inclusion level.

As shown in the biplot of OPLS model 1 (Figure 3a), hardness and *frequency sweep* parameters (E′-1 Hz, E′-10 Hz, E″-1 Hz, and E″-10 Hz) were close to each other, indicating similar changes in these parameters in response to the level of chickpea

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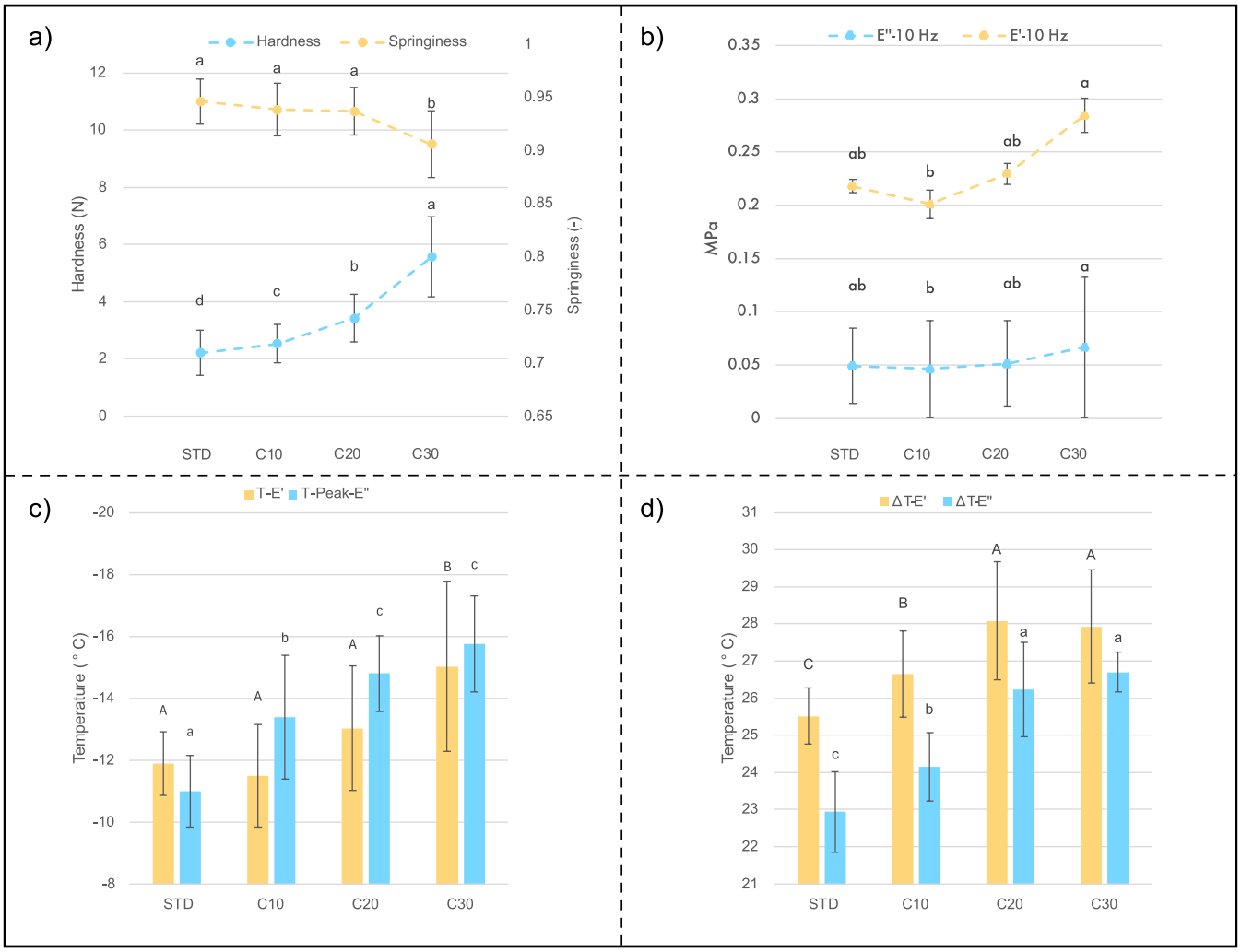

FIGURE 4 | Effect of chickpea flour content on (a) hardness and springiness; (b) E′ and E″ at 10 Hz (MPa) of bread samples; (c) temperature (°C) at which storage modulus dropped (T-E′) and temperature at the E″ prepeak (T-Peak-E″); (d) temperature range (°C) of storage modulus (ΔT-E′) and loss modulus (ΔT-E″) drops. The value shown for each chickpea inclusion level on the graph represents the mean of all corresponding stored bread samples. Different letters above the bars mean significant differences among samples (one-way ANOVA, $p \leq 0.05$).

flour inclusion. As all the *frequency sweep* variables were close to each other, they may provide the same information related to the inner relationship between the chickpea flour inclusion and the frequency-dependent viscoelastic parameters of bread. Therefore, in order to simplify the discussion, only E′ and E″ values at 10 Hz will be discussed. Storage (E′) and loss (E″) moduli showed a slight but significant increasing trend with increasing chickpea flour (Figure 4b). The increase in E′ and E″ moduli indicated an increase in bread crumb stiffness due to the presence of chickpea flour. The reasons behind these mesoscopic mechanical changes may be the same involved in crumb hardening as shown at the macroscopic level by the TPA test, which is related to the dilution of the gluten proteins and the interference of chickpea fibers and proteins on the gluten network development (Kotsiou et al. 2022). This may result in reduced bread dough expansion and crumb development, leading to higher stiffness of the sample matrix, that is, higher storage E′ and loss E″ moduli. Overall, the results indicated that the addition of chickpea flour in bread formulation negatively affected the crumb structure both at macroscopic and mesoscopic levels. Anyway, these findings should be corroborated by a sensory analysis to assess the relationship between the mechanical changes investigated at different structural scales and sensory perception.

Considering the other response variables primarily affected by the chickpea flour inclusion, those from DMA *temperature scan* parameters, T-E′, T-Peak-E″, Δ-E′, and Δ-E″ (Figures 3a S4a), are of particular interest. To better understand their relevance, it is important to note that literature indicates three possible ways to describe a thermal first order transition (e.g., ice melting), as detectable with a DMA *temperature scan* (Menard and Menard 2020). Indeed, since a transition from a rigid to a more flexible state occurs, E′ drastically decreases, and the temperature at which it drops can be measured (in our ice melting transition, this temperature was coded as T-E′). Moreover, the E″ modulus also drops during the transition, and a prepeak of E″ can be visualized before its drop due to the rearrangement of the structure, with E″ modulus increasing at a certain temperature preceding the drop. The E″ prepeak temperature can also be taken to describe a thermal transition (in our ice melting transition coded as T-Peak-E″). Furthermore, in correspondence to the

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transition, a peak of tanδ curve is also observed due to changes in the moduli ratio; the peaking temperature (in our ice melting transition coded as T-1°-tanδ) is the third way to describe a thermal transition (Menard and Menard 2020). Generally, phase transitions in bread are mostly investigated by means of the tanδ peak temperature. In our study, however, this parameter was not able to discriminate bread samples based on the chickpea flour content (Figure 3a). On the contrary, T-E′ and T-Peak-E″ parameters were found to be more sensitive to the presence of chickpea flour in bread. T-E′ (°C) and T-Peak-E″ (°C) indicate the temperature at which ice melting transition begins (Vittadini et al. 1996; Vodovotz et al. 1996). These temperatures shifted to lower values (Figure 4c) as an effect of chickpea flour inclusion in bread formulation. This effect could be attributed to the presence of higher amounts of sugars (1.9 g in 30% composite flour vs. 1.5 g in wheat flour, Table 1) and dietary fibers (4.6 g in 30% composite flour vs. 2 g in wheat flour, Table 1) in composite bread samples than in the control. Sugars, due to their hygroscopic nature, bind water molecules, reducing the amount of freezable water in the gluten-starch matrix decreasing the ice melting temperatures (Chen et al. 2012). Fibers, providing a great number of hydroxyl groups allow more water interactions throughout hydrogen bonding (Wang et al. 2002), which could also have affected the ice melting temperatures. Chickpea proteins may also have played a role in modulating water–solid interactions. Indeed, previous findings reporting lower volume and gas retention in chickpea composite bread were related to the lower vapor formation in the dough during the proofing step due to high water absorption capacity of these proteins (Aider et al. 2012).

Comparing the extent of the decrease in the ice melting temperature due to the effect of chickpea flour in bread between T-Peak-E″ and T-E′, the former was found more responsive (Figure 4c). Indeed, T-E′ (°C) was not affected by the chickpea flour content until 20% inclusion level (Figure 4c); only the substitution of wheat flour with 30% of chickpea flour resulted in a significant shift of the ice melting transition to lower temperatures. Instead, T-Peak-E″ (°C) showed significant differences already at 10% of chickpea flour if compared to STD, and it significantly increased even at 20%; however, at higher chickpea inclusion level (30%), T-Peak-E″ (°C) did not further change. This different behavior between T-E′ (°C) and T-Peak-E″ (°C) could be attributed to the different properties being studied (viscous and elastic), indicating a greater impact of chickpea flour on viscous properties (E″) than on elastic properties (E′) of the matrix.

As also previously noted and shown in Figure 3a, the temperature ranges of the storage (ΔT-E′) and loss (ΔT-E″) moduli could significantly distinguish bread formulations based on chickpea flour level, together with T-Peak-E″ and T-E′. Figure 4d shows that both parameters increased with the incorporation of chickpea flour; however, C20 and C30 were comparable. The increase of ΔT-E″ can be related to the decrease of the onset temperature transition (T-Peak-E″), which widened the temperature transition range. Indeed, C20 and C30 had the lowest T-Peak-E″ (Figure 4c) and exhibited the highest ΔT-E″. It could therefore be concluded that the temperature range in which the mechanical changes of moduli occur are useful parameters to discriminate bread samples with different amounts of chickpea flour, and thus, generally, different formulations.

4.2 | The Effect of Storage

The differences among samples due to storage time were discussed through a one-way ANOVA for mostly significative response variables based on OPLS output. The effect of storage on MC of both crumb and crust was reported in Figure S5, while the effect of storage on hardness, cohesiveness, and springiness is shown in Figure 5a. As discussed before, MC crumb and hardness were located in opposite quadrants of OPLS biplot. Indeed, as expected, these parameters were found to decrease (MC crumb) and increase (hardness) over storage, respectively. Their spatial distribution on the OPLS biplot indicated an inverse correlation between them, further confirmed by Pearson Correlation coefficient ($r = -0.989$) and in accordance with previous studies concerning bread staling. Fadda et al. (2014) and Gray and Bemiller (2003) reported that the increase in crumb hardness during storage is driven by several factors happening at different structural levels and according to different time scales; among these, macroscopic migration of water from the crumb to the crust is one of the main determinants. Bread staling is well reported also manifesting with a decrease in cohesiveness and springiness over time (Fadda et al. 2014; Fiszman et al. 2005), which agrees with our results. However, springiness values were found more constant than cohesiveness in the first days of storage. Indeed, cohesiveness decreased at each day of storage, whereas no significant differences resulted in springiness between Day 0 and Day 2.

Looking at the viscoelastic properties from the DMA *frequency sweep* test, the moduli at both 1 and 10 Hz exhibited a robust relation with hardness (Figure 5b). Specifically, the samples well described by the high relative values of these variables were bread samples of Day 7 and the C30 sample of Day 5. As can be observed from Figure 5b, moduli showed a slight but significant increasing trend during storage. Notably, between Day 0 and Day 2, there were no significant differences in any DMA *frequency sweep* parameter. This evidence could be related to starch retrogradation during bread storage. According to Wang and Sun (2002), who measured the changes of moduli in wheat bread during storage with a DMA *frequency sweep* test, E′ and E″ increased due to starch retrogradation, favored by the migration of water from gluten to starch, resulting in the hardening of the bread crumb. Indeed, during storage, starch undergoes a transition from a rubber-like to a crystallin state due to amylopectin retrogradation. This phenomenon leads to an increase in the mechanical moduli of the bread crumb (Lagrain et al. 2012; Wang and Sun 2002). The parameters obtained by the DMA *temperature scan* (T-E′ and T-Peak-E″), which describe bread viscoelastic changes associated with ice melting transition, also changed as an effect of storage (Figure 5c). However, the temperature was found to decrease for both parameters (T-E′ and T-Peak-E″) only when comparing Day 0 to Day 7. Previous studies utilizing DMA have demonstrated the impact of different water content on ice melting transition temperatures identified by *temperature scan*, indicating higher temperatures at lower water contents and narrower drop ranges (Chinachoti 1994; Chinachoti and Vodovotz 2001; Hallberg and Chinachoti 2002; Vittadini et al. 1996). According to Ribotta and Le Bail (2007) and Roos (1995), the decrease in free water resulted in an increase in solute concentration in the water phase, thereby possibly justifying the decrease in ice melting temperatures during

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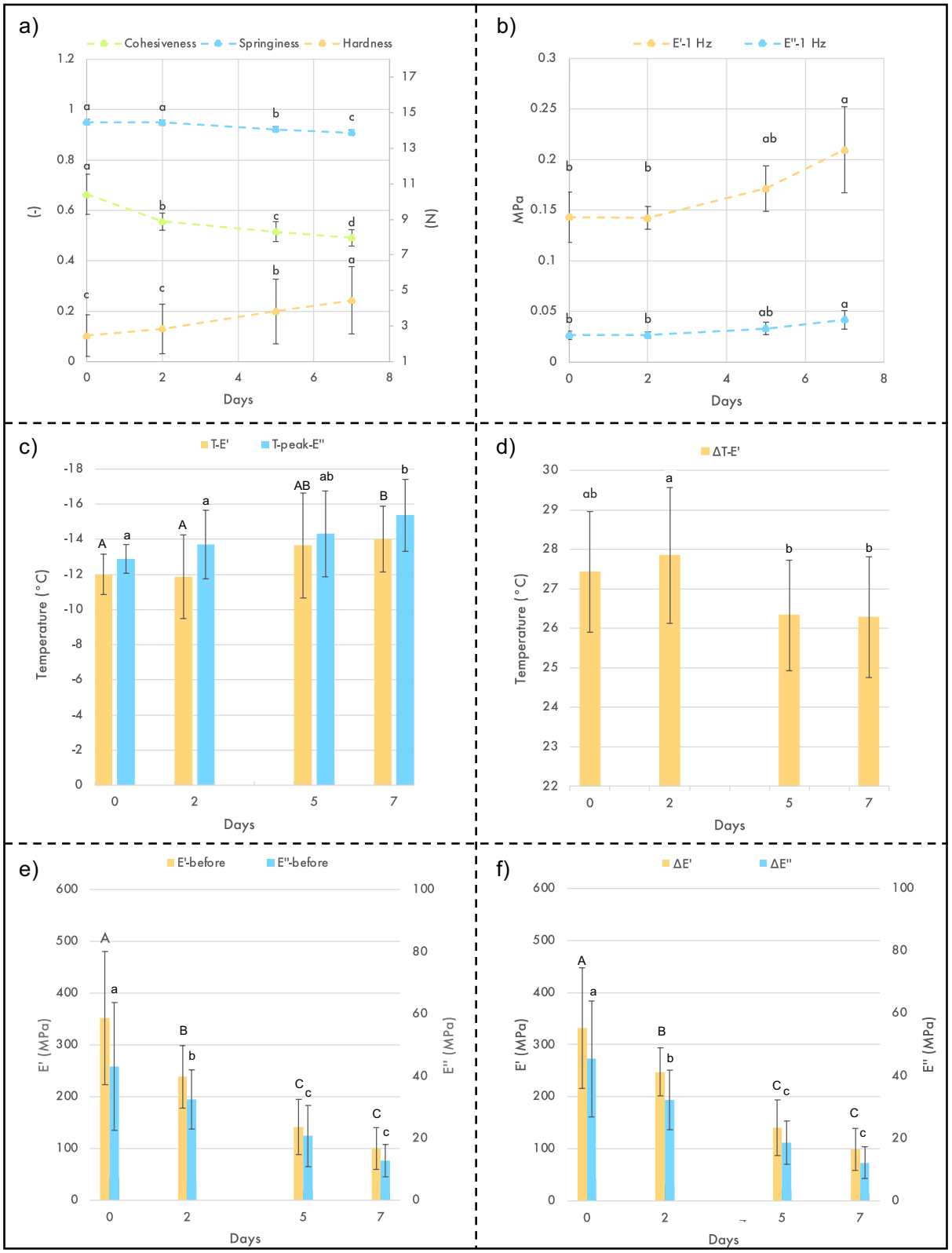

FIGURE 5 | Effect of storage time on (a) cohesiveness, springiness, and hardness; (b) E′ and E″ at 1 Hz (MPa); (c) temperature (°C) at which E′ dropped (T-E′) and at the E″ prepeak (T-Peak-E″); (d) temperature range (°C) of E′ drop (ΔT-E′); (e) values of storage (E′-before) and loss (E″-before) moduli (MPa) before the drop; (f) storage and loss moduli values difference (ΔE′; ΔE″) before and after the drop (MPa). The value shown for each storage day on the graph represents the mean of all corresponding chickpea inclusion level. Different letters above the bars mean significant differences among samples (one-way ANOVA, $p \leq 0.05$).

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storage. However, it is noteworthy that these studies covered a broad range of water contents (ranging from 49% to 14%). In contrast, our case involves less range of variability during storage, making it challenging to discern differences between Day 0 and Day 5. Moreover, the cited papers evaluated 100% wheat bread (without the addition of other flours), which may contribute to the discrepancies observed compared to our results. Figure 5d shows the temperature differences of the storage modulus (ΔT-E′) before and after the drop due to the transition. No significant differences were highlighted after 2 days, probably due to a not sufficient decrease in water content in the crumb. However, after 2 days, a significant decrease in ΔT-E′ was observed. These results were correlated with the water content in the crumb (Pearson correlation: $r = 0.841$).

As illustrated in Figure 5e, the storage (E′) and loss (E″) moduli before the drop showed a decreasing trend until the Day 5 of storage, followed by constant values until Day 7. As part of the water molecules migrated towards the crust over storage time, the remaining water molecules within the crumb are assumed to be more bound to solids and consequently less available for the phase separation in the solid-state during freezing. This results in a crumb matrix characterized by lower modulus at freezing temperatures. This observation is corroborated by the OPLS biplot (Figure 3b), where the storage and loss moduli before the drop (E′-before and E″-before) were positively correlated with the water content of the crumb (Pearson correlation: $r = 0.955$ and 0.982 for E′-before and E″-before). During storage, changes in E′-before and E″-before were directly responsible for the significant decrease in the moduli variation before and after dropping (Δ-E′ and Δ-E″; Figure 5f). As reported by Vodovotz et al. (1996), the range of moduli falling in bread decreased with decreasing water content. Our findings suggest that the range of moduli falling allows discrimination of bread at different storage time due to differences in crumb water content. However, Δ-E′ and Δ-E″ were not able to discriminate bread with different chickpea flour contents, as these variables were not included (VIP < 0.8) in the OPLS model 1 as a function of chickpea flour content. This finding suggests that these parameters are more sensitive to the water content than to other compositional factors such as starch, proteins, fats, and fiber. Indeed, the viscoelastic parameters taken from DMA *temperature scan* (E′-before, E″-before, E′-after, E″-after, Δ-E′, and Δ-E″) were not clearly affected by the presence of chickpea flour in bread formulation.

5 | Conclusion

This study investigated the impact of chickpea flour inclusion and storage time on the structural and mechanical properties of composite wheat–chickpea bread, assessed at both macroscopic (TPA) and mesoscopic (DMA) levels.

Macroscopic analysis revealed that increasing chickpea flour in bread formulation progressively hardened the crumb (+154% from 0% to 30% chickpea flour), while upper substitution levels led to a reduction in springiness (−4% from 20% to 30% chickpea flour). Additionally, storage induced typical staling-related changes, with a general increase in hardness (+100% from Days 0 to 7), and a decrease in springiness (−5% from Days 0 to 7) and cohesiveness (−25% from Days 0 to 7).

The results of the DMA *temperature scan* indicated a significant difference in T-E′ (°C), T-Peak-E″ (°C), and ΔT-E′ in relation to the chickpea flour and storage conditions, with a general decrease of ~3°C and ~2.5°C, respectively. These results confirm the possibility of detecting the migration of water from the crumb to the crust during storage with DMA, as well as the evaluation of mechanical properties as an effect of bread formulation and composition. Additionally, during storage, the ice melting transition was also characterized by a significant decrease in the ranges of moduli in which the transition occurred (Δ-E′ and Δ-E″). This may suggest that these parameters are more sensitive to the water content than to other components. However, the thermal profiles obtained allowed the characterization of the ice melting transition only. Instead, higher temperature regions proved more challenging to interpret due to the likely overlap of multiple thermal events. This complexity limits the ability to resolve transitions related to other components (e.g., starch retrogradation, protein denaturation, and water evaporation) at higher temperatures. Further studies should specifically aim to decouple the contributions of water, starch, and protein to the DMA thermograms by testing isolated components or simplified model systems and corroborating it with water mobility (^{1}H NMR) and DSC analysis. Although model systems often yield results that differ from those of complex systems, they may provide a valuable tool for improving DMA signal interpretation. Indeed, signals obtained by DMA are often difficult to interpret and highly variable, largely due to challenges in standardizing sample geometry, particularly when dealing with spongy structures such as bread.

On top of that, the application of multivariate analysis, specifically OPLS, was particularly useful in identifying which DMA variables better discriminated bread samples, emphasizing those most indicative of formulation and time-related effects. For example, of the three more common parameters used to describe the thermal transition, OPLS helped to identify that T-Peak-E″ was most highly correlated with the response variables. In the conditions tested in this study, the OPLS analysis also highlighted the limitations of tanδ, the parameter most frequently used in literature to describe the ice melting thermal transition.

Overall, the results demonstrate that DMA represents a valuable complementary analytical approach for assessing the evolution of staling and mechanical changes in chickpea-wheat bread formulations. Owing to its high sensitivity to the thermomechanical properties of food materials, this technique provides detailed information on quality changes at the mesoscopic structural level induced by both formulation and storage time, thereby offering food scientists a powerful tool to deepen their understanding of bread quality.

Author Contributions

Marcello Gigliotti: validation, formal analysis, investigation, visualization, writing – original draft. **Miriam Chiodetti:** conceptualization, methodology, validation, formal analysis, investigation, visualization, writing – review and editing. **Eleonora Carini:** conceptualization, funding acquisition, writing – review and editing, project administration, supervision, resources, methodology, validation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Parameters describing the thermal transitions selected from (a) storage modulus E′, (b) loss modulus E″, and (c) Tanδ changes upon temperature from the *temperature scan* test. **Figure S2:** Selected parameters to describe (a) storage modulus E′, (b) loss modulus E″, and (c) Tanδ changes over frequency from the *frequency sweep* test. **Figure S3:** Representative storage modulus (E′), loss modulus (E″), and Tanδ profiles for different bread at Day 0, 2, 5, and 7, obtained by means of the *frequency sweep* test. **Figure S4:** (a) OPLS loading plot of model 1, discriminating for chickpea flour content. (b) OPLS loading plot of model 2, discriminating for storage time. The response variables (yellow) were obtained from the four types of bread (STD, C10, C20, and C30) at four different storage times (0, 2, 5, and 7 days). **Figure S5:** The effect of storage on moisture content of crust and crumb (g/g). Different letters mean significant differences among samples (one-way ANOVA, $p \leq 0.05$). **Table S1:** Crust and crumb moisture content, and TPA (hardness, cohesiveness, and springiness) results. Data are reported as mean ± standard deviation. **Table S2:** Dynamic mechanical analysis (DMA) results. Data are reported as mean ± standard deviation.

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