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Viscoelastic Properties of Biscuit Doughs with Different Lipidic Profiles Fortified with a Casein Hydrolysate

Ricardo Troncoso [1,*], Ana Torrado [1], Nelson Pérez-Guerra [1] and Clara A. Tovar [2]

[1] Department of Analytical and Food Chemistry, University of Vigo, 32004 Ourense, Spain
[2] Department of Applied Physics, University of Vigo, 32004 Ourense, Spain
* Correspondence: ricardo.troncoso@uvigo.es

Abstract: The effects of using a hard (artisanal) margarine (which has a higher lipidic and lower aqueous contents) and using a soft (commercial) margarine (which has a lower lipidic and higher aqueous contents), along with a casein hydrolysate, on the rheological properties of different biscuit short doughs were examined. The characteristic parameters in the linear viscoelastic range (LVER) were analysed by stress sweep at 6.3 rad/s and 25 °C. The two margarines showed similar values of strain amplitude (γ_{max}), but the hard margarine exhibited a significantly higher firmness in the LVER, as expected. An analogous result was found for the biscuit doughs made with hard margarine and soft margarine. The addition of a casein hydrolysate (CH) to both biscuit doughs produced an increase in the loss factor, indicating a loss of the solid-like character in the dough networks. Nevertheless, a different trend in the consistency of the dough, which depended on the type of margarine, was found. While, after adding CH, the dough made with soft margarine showed a significant reduction in rigidity, the dough with hard margarine exhibited an increased firmness upon CH addition. Yield stress tests showed that CH facilitates the transition from elastic to plastic deformation at the yield point more intensely in the dough with soft margarine.

Keywords: biscuit dough; casein hydrolysate; margarine; viscoelasticity; yield point; rheology

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1. Introduction

Biscuits are cereal-based products with a low water content (below 5% after baked), made from flour and containing variable amounts of fat and sugar, depending on the type of biscuit. Specifically, short dough biscuits contain high levels of fat and sugar (typically 30–40%), and the dough obtained before baking exhibits a soft and cohesive texture [1].

The network of a biscuit short dough can be described as a multiphase composite comprising a heterogeneous solid matrix composed of a polymeric complex (proteins and small carbohydrates from flour, sugar, and water), a filler phase (starch), and margarine as fatty phase. The margarine is partially emulsified and cross-linked to the main matrix, resulting in an irregular and heterogeneous three-dimensional network with gel properties [1,2]. Therefore, the fat in biscuit dough plays a crucial structural role, binding all components of the blend and yielding a soft dough with appropriate handling and processing characteristics. Additionally, fat facilitates air incorporation, reduces the hardness and elasticity of the dough, and may act as a lubricant during mixing, preventing the formation of the gluten network [3]. Generally, if the fat is firmer than the dough, it may cause the dough to break during mixing. Conversely, if the fat is softer than the dough, it could migrate into the dough [4]. Among the fats used for biscuits, margarine is commonly employed because it facilitates dough handling. Margarine is a water-in-oil emulsion that contains dispersed water droplets in an oily medium. The latter consists of triacylglycerols containing unsaturated and saturated fatty acids (solid fat) [4,5]. The physical functionality of margarine is influenced by the ratio of saturated fatty acids (SFA) in triacylglycerols, the fatty acids chain length, and the water content, among other factors.

Therefore, the higher the SFA content, the harder the margarine becomes, as these fatty acids can be ordered (aligned) and packed [5], resulting in a firmer and more consistent structure. Accordingly, using margarine with a higher SFA and a lower water content is expected to increase the solid-like behaviour of dough. In fact, a biscuit dough made with margarine containing a higher SFA level (palm kernel-based fat) exhibited greater dough hardness and less cohesiveness compared to a biscuit dough containing margarine with a lower SFA level (palm-based fat) [6]. Ismail et al. (2018) [7] also reported that harder dough resulted when a fat with a higher solid fat content (solid shortening) was used instead of fluid shortening. Tarancón et al. (2015) [8] replaced biscuit fat with a sunflower oil–water–cellulose emulsion, reducing SFA content, and resulting in a decrease in the viscoelastic moduli. Mamat and Hill (2014) [3] reported that using palm mid-fraction fat, with a higher solid fat index (higher SFA content) than palm oil or palm olein, led to a harder dough that required greater strength to reach the rupture point compared to the dough made with a lower solid fat index (palm oil or palm olein). In contrast, dough made using fat with a lower SFA content (palm olein) exhibited a softer texture that required less force to be compressed. Thus, the rheological characteristics of biscuit doughs are essentially dependent on the relative firmness of bakery fat and determine the processability and quality of the biscuits [3,9].

On the other hand, biscuit doughs could be enriched with proteinaceous ingredients to facilitate dough handling and increase the nutritional value of the final product. Specifically, the incorporation of a casein hydrolysate (CH) [10] can be used to address the deficit of flour proteins in sulfur-containing essential amino acids. Protein malnutrition is a significant issue in developing countries, especially concerning the deficit in essential amino acids [11]. Therefore, the protein fortification of biscuits is interesting, since they are widely accepted foods [12] with a long shelf life. Caseins are remarkable candidates for protein fortification considering their well-known nutritional value due to their balanced essential amino acid composition and high content of available calcium and phosphorous. Additionally, caseins exhibit interesting techno-functional properties due to their amphiphilic character. This characteristic enables them to act as surfactants, emulsifiers, thickeners, and foaming and water-binding agents in flour doughs [13]. A casein hydrolysate (CH) is even more suitable than whole caseins because its solubility is increased and the allergic reactions are prevented, while maintaining the nutritional value and some of the functional properties [14]. Moreover, CH is also interesting due to its reported mineral binding capacity, which promotes the absorption of calcium [15].

The addition of a casein hydrolysate may induce rheological changes in the biscuit dough that could alter the mechanical manipulation during processing. For instance, empirical analysis has shown that the addition of casein or CH decreases dough extensibility and pasting properties (viscosity), resulting in a weaker dough structure [16]. Additionally, the addition of 5–10% sodium caseinate (NaCas) reduced the dough hardness [17]. In contrast, Sert et al. (2016) [13], reported a significantly higher dough consistency upon NaCas addition.

However, there are no research studies using fundamental rheological tests to explore the rheological role of CH within biscuit doughs with different lipidic compositions. Although casein hydrolysates can be used as ingredients to improve the nutritional value of food products [12], these compounds might also be used as novel agents to enhance the processing steps and viscoelastic properties of biscuit dough. In this sense, it must be considered that the nutritional and functional value of food components does not depend solely on chemical composition. The way in which molecules interact with each other and generate food micro- and macrostructures can affect the extent and the efficiency of gastrointestinal digestion and, hence, impact the bioavailability of the food components [18]. Therefore, the structural changes produced by a combination of different ingredients must be considered for a further nutritional evaluation of the final food products.

Thus, the aim of this work was to examine the combined effect of two ingredients (margarine and a casein hydrolysate) on the fundamental rheological properties of a biscuit

short dough. Two types of margarines with different fatty acid profiles and water contents were tested to explore the potential interactions between the fatty ingredient and the casein hydrolysate in biscuit short dough.

2. Materials and Methods

2.1. Ingredients of the Biscuit Doughs

The basic ingredients used for the preparation of biscuit dough samples were wheat flour (Haribericas XXI S.L., Andalucía, Spain) with 73% starch, 10.3% protein, 13.6% water, 1.3% fat, and 0.59% ash; sugar (Sociedad Cooperativa General Agropecuaria ACOR, Valladolid, Spain); refined dry salt (Salinas del Odiel S.L., Huelva, Spain); and tap water. Two margarines with different lipidic profile were used: a commercial "soft" margarine (SM) (Vandermoortele, Izegem, Belgium) with 40% water and 60% lipids (with 17% SFA), and an artisanal "hard" margarine (HM) for bakery products (Gracomsa Alimentaria S.A., Valencia, Spain) with 18% water and 80% lipids (with 37% SFA). The commercial product Hyvital Casein Phosphopeptides (FrieslandCampina Domo, Amersfoort, The Netherlands) was used as the source of the casein hydrolysate (CH) for biscuit doughs. The product consists of a CH with a 17% degree of hydrolysis and an average molecular weight of 600 Da, of which 24% are casein phosphopeptides (CPPs). The product contains 91.3% protein, 5.0% moisture, and 6.2% ash. Regarding the amino acid composition, Hyvital Casein Phosphopeptides contain glutamic acid (209 mg/g) and cysteine (3 mg/g) among others (https://www.frieslandcampinaingredients.com/, accessed on 10 December 2017).

Four formulations of biscuit doughs were prepared with all combinations of soft and hard margarine, with and without the casein hydrolysate (Table 1).

Table 1. Formulations used in biscuit dough samples: Biscuit dough with soft margarine (SD), CH-fortified biscuit dough with soft margarine (SD-C), biscuit dough with hard margarine (HD), and CH-fortified biscuit dough with hard margarine (HD-C) (% refers to flour content).

Dough Ingredients	SD	SD-C	HD	HD-C
Wheat Flour (g/100 g)	46.02 (100%)	41.99 (100%)	43.92 (100%)	41.97 (100%)
Margarine (g/100 g)	32.34 [1] (70%)	32.12 [1] (76%)	33.57 [2] (76%)	32.13 [2] (77%)
Sugar (g/100 g)	19.27 (42%)	19.20 (46%)	20.04 (46%)	19.21 (46%)
Water (g/100 g)	2.21 (4.8%)	2.21 (5.3%)	2.30 (5.2%)	2.20 (5.2%)
Salt (g/100 g)	0.16 (0.4%)	0.16 (0.4%)	0.16 (0.4%)	0.16 (0.4%)
CH (g/100 g)	-	4.33 (10%)	-	4.33 (10%)

[1] margarine with 17% SFA and 40% water. [2] margarine with 37% SFA and 18% water.

2.2. Biscuit Dough Samples Preparation

A two-stage mixing procedure was employed, as it is the most appropriate method for producing biscuit short dough [1]. Initially, sugar, margarine, and water were mixed in a planetary kneader (Sammic BM-5, Leicester, UK) for 10 min at 275 rpm and room temperature to dissolve the sugar and form an emulsion with the fat, until obtaining a semi-stiff white cream ('cream-up' stage). Next, in the 'dough-up' stage, the flour and salt were added to the blend and mixed for 6 min at 275 rpm to achieve a homogeneous mixture. When applicable, CH was previously mixed with the flour and salt in another bowl until a homogeneous colour was obtained, and then, the mixture was added to carry out the second step. Finally, a soft and viscoelastic dough was obtained. Measurements were conducted over the following 5 days, keeping the chilled samples packed in the refrigerator (4 °C) (QC 421- Balay, Zaragoza, Spain) to take samples during the experiment. Considering the experimental uncertainty, no significant differences ($p > 0.05$) were found between replicates from the same sample within those 5 days.

2.3. Rheological Tests

Oscillatory measurements were performed using a RS600 rheometer (Haake, Thermo Electron Karlsruhe, Germany), equipped with a 20 mm parallel-plate geometry and a

gap of 1 mm. To ensure thermal and mechanical equilibrium, samples were rested for 15 min before measurement. Throughout all tests, the temperature of the lower plate was kept at 25.0 ± 0.1 °C, using a fluid circulating bath (Haake SC 150 Thermo Scientific, Karlsruhe, Germany). To prevent sample drying during measurement, a steel solvent trap was employed.

2.3.1. Amplitude Sweeps

Amplitude sweeps were conducted to identify the linear viscoelastic region (LVER) at a frequency of 1 Hz. The shear stress range applied was 20–200 Pa, increasing in 250 steps with a maximum strain of 100%. Stress sweep data were utilised to determine the stress/strain limit values of the LVER at which the complex modulus (G*) began to deviate by 10% from the initial value [19]. This measurement point provides the limit values of stress (σ_{max}) and strain (γ_{max}) amplitudes. The experiments were repeated five times.

The linear representation of Equation (1) enables the simultaneous visualisation and comparison of two key variables: (1) the extent of the maximum strain-range of the LVER (γ_{max}), and (2) the relative inclination of the straight line (slope "*a*") based on the different compositions of both margarines (Equation (1)).

$$\sigma = a \cdot \gamma + b \quad (1)$$

The slope "*a*" (in Pa) measures the resistance to the total (elastic and viscous) deformation, indicating the firmness of the network (gel strength) [20]. The independent term "*b*" (in Pa) represents the stress at the initial state ($\sigma = \sigma_0$).

Furthermore, Equation (1) can be approached as a definite integral (Equation (2)), extending from the initial strain (γ_0) to the maximum strain amplitude (γ_{max}):

$$E = \int_{\gamma_0}^{\gamma_{max}} (a \cdot \gamma + b) d\gamma \quad (2)$$

The E value (in J/m^3) represents the area under the straight line of σ vs. γ in the LVER. It determines the energy stability of the gel network in the LVER [20].

2.3.2. Frequency Sweeps

Frequency sweeps were conducted at increasing frequencies over a range between 0.1 and 10 Hz (27 steps). This oscillatory test was performed while maintaining the sinusoidal strain at a constant strain (0.1%) within the LVER. The storage modulus (G′) and loss modulus (G″) were recorded. These moduli can be fitted to the power-law equation (Equations (3) and (4)).

$$G' = G_0' \cdot \omega^{n'} \quad (3)$$

$$G'' = G_0'' \cdot \omega^{n''} \quad (4)$$

where: G_0' and G_0'' are the storage and loss modulus, respectively, at 1 rad/s. The n' and n'' exponents indicate the influence of the angular frequency (ω) on G′ and G″, respectively [20].

2.3.3. Yield Stress Test

The yield point was determined through a rotatory test by applying a continuous stress ramp from 20 to 600 Pa at 25 °C (100 steps) over 10 min. This test provides two characteristic parameters: critical viscosity (μ_c) and critical stress (σ_c). These parameters indicate the transition point between reversible and irreversible deformation [21].

2.4. Statistical Analysis

Rheological data are presented as the mean values of seven replicates and were tested with expanded uncertainty limits (EUL) representing the maximum and minimum deviation from the respective mean value. Trends were considered significant when the

means of compared sets differed at $p < 0.05$ (Student's *t*-test). This statistical method was validated using SPSS statistics 29.0.2 software [20].

3. Results and Discussion

3.1. Overview

An initial viscoelastic study of the LVER and mechanical spectra for the fatty ingredient (soft and hard margarines) was conducted to understand the influence of water content and the different lipidic profiles of the fatty acids on the viscoelastic properties of margarines. Then, the viscoelastic behaviour of the biscuit doughs made with each kind of margarine was compared. Finally, the combined effect of the casein hydrolysate and and soft or hard margarine on the rheological properties of the fortified biscuit dough was investigated.

3.2. Linear Viscoelastic Range (LVER)

3.2.1. LVER for Soft/Hard Margarines (SM/HM) and Biscuit Doughs (SD/HD)

The LVER can be observed through the graphical representation of the linear relationship between the experimental values of stress (σ) vs. strain (γ) (Figure 1). This graph extends from the initial strain ($\gamma = \gamma_0$) to the maximum (critical) strain ($\gamma = \gamma_{max}$), called strain amplitude [22]. Regarding the margarines, HM exhibited higher values for the "*a*" and "*b*" parameters and a slightly lower γ_{max} compared to SM (Table 2). These parameters indicate the greater consistency of HM compared to SM, which could be attributed to its higher lipid content, and particularly, a greater amount of SFA, and lower water content, resulting in a harder margarine. Additionally, fat crystals derived from saturated fats may form a heterogeneous three-dimensional network that binds the liquid phase (water and oil) [23]. This irregular network in HM could explain the higher experimental uncertainties in the phase angle (δ) and the strain amplitude (γ_{max}) (Table 2).

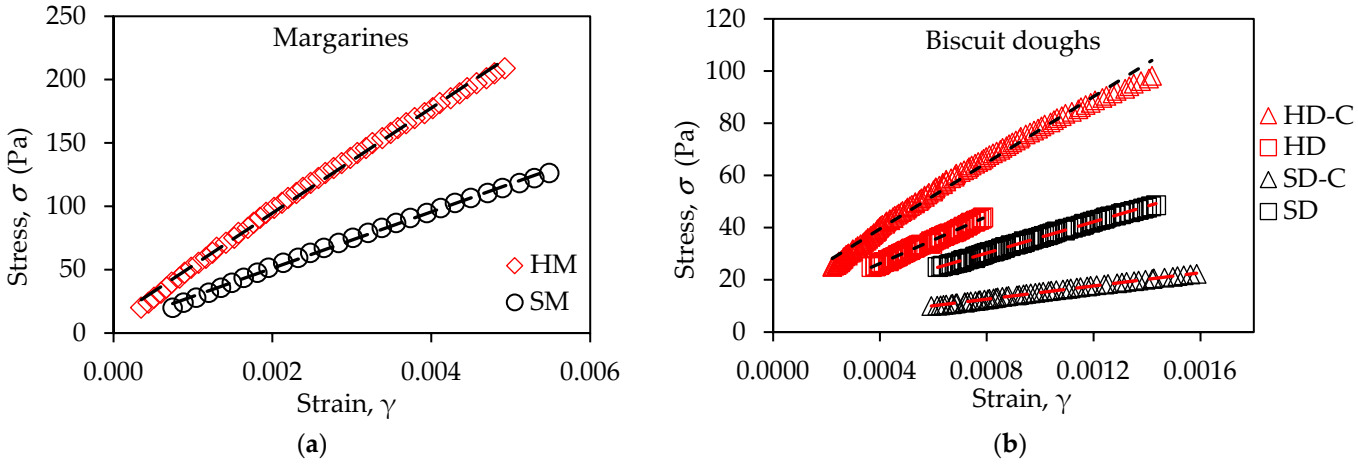


Figure 1. Stress vs. strain diagrams for: (**a**) soft margarine (SM) and hard margarine (HM); (**b**) biscuit doughs made with soft (SD) and hard margarine (HD), CH-fortified biscuit dough with soft margarine (SD-C), and CH-fortified biscuit dough with hard margarine (HD-C). Frequency = 1 Hz and T = 25 °C.

When the soft and hard margarines were added to the biscuit doughs, noticeable differences in the slope "*a*" were observed (Figure 1b). The "*a*" value was 50% higher for the dough made with hard margarine (HD), indicating the ability of hard margarine to pack the dough, resulting in a tighter network. This was also evidenced by the significant decrease ($p < 0.05$) in conformational flexibility (lower γ_{max}) in HD compared to SD (Table 2). Similarly, it is reasonable to attribute the greater stiffness and lower deformability of the composite matrix HD to the greater amount of saturated fats in hard margarine, which is more than twice that in soft margarine). In this case, soft margarine provided a structural benefit to the composite network (SD) by reducing excessive rigidity and improving the conformational flexibility (higher γ_{max}) of the SD dough [24].

Table 2. Fit parameters *a* and *b* (Equation (1)) and experimental viscoelastic data: reticular energy (*E*, Equation (2)), phase angle (δ), and strain amplitude (γ_{max}) in the LVER at 1 Hz. T = 25 °C.

Sample	*a* (kPa)	*b* (Pa)	r^2	*E* (J/m^3)	δ (°)	γ_{max}
SM	22.23 ± 0.20 ^{a}	6.65 ± 0.66 ^{a}	0.998	0.361 ± 0.034 ^{a}	10.60 ± 0.25 ^{a}	0.00549 ± 0.00051 ^{a}
HM	41.42 ± 0.35 ^{b}	11.86 ± 0.98 ^{b}	0.997	0.560 ± 0.260 ^{a}	11.70 ± 2.10 ^{a}	0.00493 ± 0.0023 ^{a}
SD	29.95 ± 0.13 ^{c}	6.32 ± 0.13 ^{a}	0.998	0.030 ± 0.004 ^{b}	24.58 ± 0.41 ^{b}	0.00143 ± 0.00019 ^{b}
HD	44.95 ± 0.31 ^{d}	8.29 ± 0.18 ^{c}	0.997	0.014 ± 0.003 ^{c}	19.60 ± 1.20 ^{c}	0.00079 ± 0.00015 ^{c}
SD-C	12.67 ± 0.07 ^{e}	2.49 ± 0.07 ^{d}	0.998	0.016 ± 0.002 ^{c}	27.44 ± 0.61 ^{d}	0.00159 ± 0.00018 ^{b}
HD-C	63.42 ± 0.57 ^{f}	14.14 ± 0.40 ^{e}	0.989	0.079 ± 0.023 ^{d}	22.90 ± 3.20 ^{bc}	0.00142 ± 0.00042 ^{b}

Mean values ± standard deviation. Different letters (superscripts a–f) in the same column indicate significant differences ($p < 0.05$) among samples.

The *E* value was significantly lower in the HD compared to the SD (Table 2). This result suggests that the substantial loss of conformational flexibility caused by the hard margarine in the HD compared to the SD resulted in a significant ($p < 0.05$) reduction in the energy stability of the composite network. Therefore, a firmer and more irregular structure, possibly due to the diverse size and shape of the fat crystals in saturated fats, produced a more shear sensitive (less shear-stable) network (HD), requiring less energy to reach the limit strain of the LVER.

3.2.2. Effect of the Casein Hydrolysate on the LVER of Soft/Hard Biscuit Doughs

Incorporating a casein hydrolysate (CH) into the doughs resulted in different trends in firmness depending on the type of margarine used. Adding CH to the soft dough (SD-C) notably decreased the *a*-slope compared to the soft dough without CH (SD) (Figure 1b). While both doughs (with and without CH) showed similar conformational flexibilities (γ_{max}) (Table 2), the strong decrease in *a* parameter indicated a notable loss of stiffness in the SD-C compared to the SD. These findings were also reflected in the significantly ($p < 0.05$) lower E value for the SD-C compared to the SD (Table 2). These results could be attributed to the high content of casein phosphopeptides and glutamic acid (charged components) in CH, which might increase the electrostatic repulsive forces and weaken the hydrophobic interactions in the composite network. The higher moisture content and lower saturated fat in the soft margarine would produce a particular wet environment in the composite matrix, inducing new ion–dipole interactions among ionic peptides and water molecules in the SD-C. Hence, the SD-C network would result in a more hydrated structure associated to a net softening in the SD-C compared to the SD. This is consistent with the significantly ($p < 0.05$) higher phase angle (δ) value, which was 12% higher in SD-C compared to SD (Table 2). The higher δ indicates a more open network with less energy cohesiveness in the SD-C vs. the SD dough [24].

Conversely, in the presence of hard margarine, the firmness (*a*-slope), conformational flexibility (γ_{max}), and reticular energy (*E*) of the dough (HD-C) significantly increased ($p < 0.05$) after the addition of casein hydrolysate (Table 2). These results indicate a net reinforcement produced in the composite network in the HD-C compared to the HD. This effect could be attributed to the high ability of the casein peptides to interact with and bind water. Given the very low amount of water in the complex HD-C system, it is reasonable to assume that the soluble casein peptides are confined within the water droplets. Meanwhile, the hydrophobic casein peptides could reinforce the apolar interactions in the whole network. As a result, a net strengthening may be induced in the dough network of the HD-C compared to the HD. Additionally, considering the amphiphilic character of CH [10], these peptides could act as linking agents between the hydrophilic and hydrophobic components of the HD-C network. Thus, an extended and heterogeneous multiphasic complex (fat–protein–starch–water–sugar) may be formed in the HD-C compared to the HD. This net reinforcement of the molecular composite in the HD-C reflects a peculiar intermolecular coupling between the hydrophilic (starch–hydrophilic amino acids–sugar–water) and hydrophobic compounds (fat–hydrophobic amino acids). All these structural effects could

be promoted by the high content of SFA and the low amount of the available water in the hard margarine, enhancing the internal order within this network of molecular aggregates.

3.3. Mechanical Spectra

3.3.1. Mechanical Spectra for Soft/Hard Margarines (SM/HM) and Biscuit Doughs (SD/HD)

Figure 2a shows the mechanical spectra of both the SM and the HM margarines, wherein it is exhibited that G′ exceeded G″ across the entire frequency range. These values of G′ and G″ indicate that the crystalline phase of saturated fats in both margarines formed a three-dimensional network that maintains the water-in-oil emulsion in a gel-like structure. Naturally, the HM exhibited higher viscoelastic moduli than the SM across the range of all frequencies, with a lower frequency dependence indicating stronger consistency and a gel-like behaviour. This result could be attributed to the lower moisture content and higher amount of SFA in the HM compared to the SM, resulting in a more packed emulsified gel-network. The transient nature (time dependence) of these gels was evidenced in the positive (n') and negative (n'') exponents (Table 3). This result shows that at low frequencies ($\omega < 0.001$), a gel-to-sol transition will occur. This phase transition is faster in the SM, as evidenced by the higher relative difference between n' and n'' (33%) compared to hard margarine (27%) (Table 3).

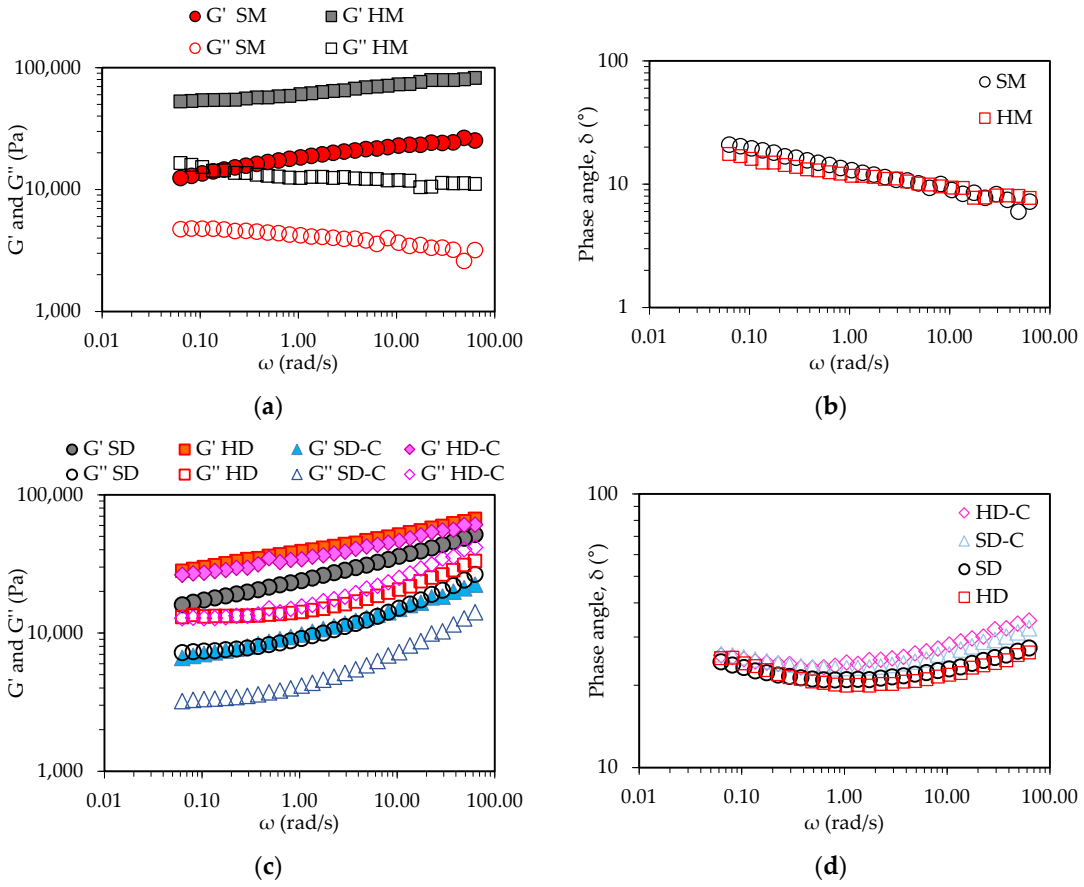


Figure 2. Mechanical spectra in terms of elastic (G′) and viscous (G″) moduli: (**a**) soft (SM) and hard (HM) margarines. Mechanical spectra in terms of phase angle (δ): (**b**) soft (SM) and hard (HM) margarines. Mechanical spectra in terms of elastic (G′) and viscous (G″) moduli: (**c**) biscuit doughs with soft (SD) and hard margarine (HD), CH-fortified with soft margarine (SD-C), and CH-fortified with hard margarine (HD-C). Mechanical spectra in terms of phase angle (δ): (**d**) biscuit doughs with soft margarine (SD), with hard margarine (HD), CH-fortified with soft margarine (SD-C), and CH-fortified with hard margarine (HD-C). T = 25 °C.

Table 3. Fit parameters (mean ± expanded uncertainty limit (EUL) from Equations (3) and (4) (T = 25 °C) of soft margarine (SM), biscuit dough with soft margarine (SD), CH-fortified biscuit dough with soft margarine (SD-C), hard margarine (HM), biscuit dough with hard margarine (HD), and CH-fortified biscuit dough with hard margarine (HD-C).

Sample	G_0' (kPa)	n'	G_0'' (kPa)	n''
SM	17.8 ± 5.0 ^{a}	0.103 ± 0.003 ^{a}	4.18 ± 0.96 ^{a}	–0.069 ± 0.005 ^{a}
HM	62 ± 17 ^{b}	0.069 ± 0.002 ^{b}	13.1 ± 2.6 ^{bc}	–0.050 ± 0.004 ^{b}
SD	24.4 ± 3.3 ^{a}	0.166 ± 0.002 ^{c}	10.0 ± 1.3 ^{b}	0.188 ± 0.009 ^{c}
HD	39.1 ± 5.1 ^{d}	0.122 ± 0.001 ^{e}	15.8 ± 1.7 ^{c}	0.131 ± 0.010 ^{e}
SD-C	10.0 ± 1.2 ^{c}	0.177 ± 0.003 ^{d}	4.66 ± 0.61 ^{a}	0.214 ± 0.011 ^{d}
HD-C	35.4 ± 4.4 ^{d}	0.123 ± 0.003 ^{e}	17.1 ± 3.2 ^{c}	0.178 ± 0.009 ^{c}

Different letters (superscripts $^{a–e}$) in the same column (experimental parameters) indicate significant differences ($p < 0.05$) among samples.

Both types of margarines induced noticeable viscoelastic differences in the mechanical spectra for the SD and the HD doughs. Similar to the margarines, higher values of the viscoelastic moduli (greater consistency) were observed in the dough made with hard margarine (HD), regardless of frequency (Figure 2c). This trend was evidenced by the significantly ($p < 0.05$) higher values of G_0' and G_0'' for the HD compared to the SD (Table 3). These data highlight the synergic interaction between the hydrophilic and hydrophobic phases, forming a consistent three-dimensional network in biscuit dough, with a greater network density in the HD [25]. Furthermore, the HD dough exhibited significantly lower n' and n'' values ($p < 0.05$) than SD dough (Table 3). This result indicated a reduced frequency dependence of both G′ and G″ moduli. Therefore, the HD network exhibited a more time-stable three-dimensional network, which is equivalent to a more permanent gel [24]. This observation was evidenced by the slightly positive difference between n'' and n', specifically 7% for HD and 12% for SD (Table 3). The lower water content and the higher lipid content of the hard margarine probably contribute to the higher temporal stability of the dough network (HD).

3.3.2. Influence of CH on Mechanical Spectra of Biscuit Doughs

Figure 2c indicates that the SD-C exhibited lower elastic and viscous moduli across all frequency range compared to the SD. This trend suggests that CH could induce some structural disruption in the multiphase complex (fat–protein–water–starch–sugar), thereby reducing the net connectivity and the subsequent proportion of elastically active compared to dangling chains [26]. This was also evidenced by the higher value of tanδ in SD-C dough (0.477 ± 0.017) compared to the SD (tanδ = 0.420 ± 0.012). These results are in agreement with those of Buresová et al. (2016) [27], who reported a decrease in G′ and G″ moduli in the gel network after the addition of sodium and calcium caseinate to a rice-buckwheat dough. Furthermore, the significant ($p < 0.05$) increase in the n' and n'' exponents indicate a greater frequency dependence of G′ and G″ in the SD-C compared to the SD (Table 3). This fact suggests a reduced time stability in the CH-fortified soft biscuit dough.

However, for the dough made with hard margarine, the addition of the CH did not significantly ($p > 0.05$) change the values of the G_0' and G_0'' parameters. Consequently, the complex modulus (G*) was similar and statistically indistinguishable in both cases, G* = 39.3 ± 5.1 kPa for HD-C and 42.2 ± 5.0 kPa for HD. Considering that G* provides a measurement of the viscoelastic gel strength [19], these data indicate that the addition of CH did not practically affect the gel structure in the biscuit dough made with hard margarine (HD-C).

The different effect of the casein hydrolysate in the HD observed from stress sweeps (greater strengthening of HD-C vs. HD) compared to frequency sweeps (similar strength in both HD-C and HD) could be explained by the different physical principles of measurement in both tests [22]. In stress sweeps, the frequency value is constant,; thus, the same time scale was applied to all samples. In this case, the key variable is the increased stress/strain,

which belongs to the structural domain [20]. However, in frequency sweeps, the key variable is the frequency; therefore, the mechanical spectra give the values of G′ and G″ at different time scales (temporal domain) under low fixed constant stress. For that reason, the mechanical spectrum is considered the fingerprint of samples [22]. In this case, the stabilising effect of the casein hydrolysate on the dough made with hard margarine was evidenced in the similar n' values for HD-C vs. HD (Table 3). This result indicates the same time dependence on the elastic modulus, which is equivalent to the similar degree of permanence in connectivity among the elastically active chains in both the HD-C and the HD networks [26]. Therefore, the potential surfactant role of CH likely influences the formation of a macro-molecular complex between the hydrophilic and hydrophobic components in the HD-C. This enhances the net coupling in the multiphase system (lipid–proteins–starch–sugar–water), leading to a temporally stable gel network.

3.4. Study of the Yield Point

Comparison between Hard and Soft Biscuit Dough: Influence of CH on the Yield Point

Figure 3 displays the viscosity values for the four biscuit doughs under increasing stress, where the values of critical viscosity (μ_c) and critical stress (σ_c) can be inferred from the curve maximum. These parameters characterise the rheological transition from reversible (elastic) to irreversible (plastic) deformations, based on the potential energy barrier between inter-particle interactions [21]. Consequently, for $\sigma > \sigma_c$, there is a decrease in the number and size of crosslinks in the network, resulting in mobile molecular fragments that facilitate inner molecular motion, as evidenced by the progressive decrease in apparent viscosity (Figure 3).

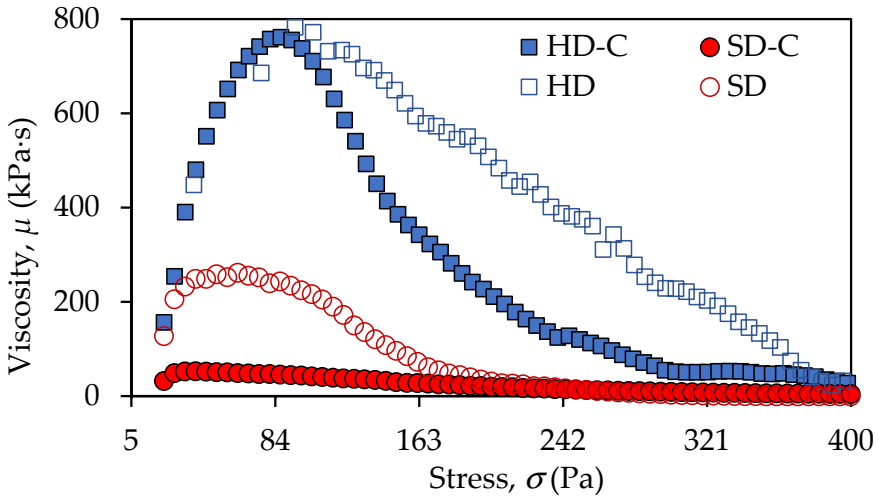


Figure 3. Apparent viscosity with increasing shear stress of biscuit doughs with soft margarine (SD) and with hard margarine (HD), CH-fortified biscuit dough with soft margarine (SD-C), and CH-fortified biscuit dough with hard margarine (HD-C). T = 25 °C.

The HD presented a significantly ($p < 0.05$) higher critical viscosity ($\mu_c = 783 \pm 169$ kPa·s) and critical stress ($\sigma_c = 95 \pm 14$ Pa) compared to the SD ($\mu_c = 262 \pm 28$ kPa·s; $\sigma_c = 63.3 \pm 6.9$ Pa). These data suggest that HD has greater "mechanical power" against structural collapse than SD (Figure 3). This result agrees with the greater consistency observed in amplitude sweeps, where the HD showed a higher "*a*" slope and a lower δ value compared to the SD (Table 2).

After adding CH to the soft biscuit dough (SD-C), the values of $\mu_c = 53 \pm 20$ kPa·s and $\sigma_c = 40 \pm 4$ Pa were significantly ($p < 0.05$) lower than those of SD ($\mu_c = 262 \pm 28$ kPa·s; $\sigma_c = 63.3 \pm 6.3$ Pa). Thus, CH caused a significant drop in the critical parameters, suggesting a loss of connectivity in the multiphase composite of biscuit dough (SD-C), resulting in a weaker gel network. This result highlights the thinning role commonly associated with CH in other food doughs [10,28]. However, when CH was added to the dough with hard

margarine (HD-C), no significant ($p > 0.05$) differences were found in the corresponding critical parameters: $\mu_c = 762 \pm 178$ kPa·s and $\sigma_c = 87.1 \pm 2.6$ Pa in the HD-C compared to the HD ($\mu_c = 783 \pm 169$ kPa·s; $\sigma_c = 95 \pm 14$ Pa). This result suggests a stabilising role of CH on the composite network (HD-C), consistent with the similar complex moduli (G*) analysed in the mechanical spectrum (Table 3). In contrast, the faster rate of decrease in the apparent viscosity beyond the yield point in the HD-C compared to the HD (Figure 3) indicates the higher viscous component (higher δ value) observed in the HD-C compared to the HD (Table 2).

4. Conclusions

All biscuit doughs exhibited a sustained solid-like nature even at low frequencies. The principal rheological differences among biscuit doughs were dependent on the lipidic composition and water content of the soft margarine or the hard margarine used in their elaboration. Hard margarine produced a stiffer and less deformable biscuit dough, which was more time-stable compared with soft biscuit dough. Soft margarine contributed to structural improvements in the composite network by reducing the excessive rigidity, enhancing the conformational flexibility and energy stability of the biscuit dough.

The addition of a casein hydrolysate to the biscuit doughs produced varying effects on the rheological properties of doughs depending on the margarine used. In the biscuit dough made with soft margarine, the hydrolysate notably increased the viscous component of the gel network, reducing the net connectivity within the composite network. However, in the biscuit dough made with hard margarine, the casein hydrolysate exhibited a higher gel strength, conformational flexibility, network energy, and stability time in the gel network. Accordingly, the casein hydrolysate may act as a stabilising agent in both the structural and the temporal domains when added to the biscuit dough made with hard margarine.

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