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Improving the Technological and Nutritive Properties of Gluten-Free Bread by Fresh Curd Cheese Enrichment

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Abstract: Replacing wheat flour in the breadmaking process is a technology challenge since the elimination of gluten has a strong influence on bread quality. Proteins addition are often used to form a protein network capable of mimicking gluten-like structure, giving to dough a foaming support. This study aimed to evaluate the potential of denatured whey proteins coming from fresh curd cheese addition, to strengthening gluten-free dough structure, enhancing the breadmaking performance. Curd cheese additions were tested (5% up to 20%, weight/weight) and the effect on dough rheology behavior and bread quality was evaluated. Findings obtained revealed that the technology and nutritional properties of the bread can be enhanced by curd cheese addition, and such effects should be related to the composition and functionality of denatured whey proteins. Considering higher levels of curd cheese (20%) tested, improvements on bread quality was observed, leading to a considerable increase in bread volume (73%), softness (65%), with a significant reduction on staling kinetics (70%), comparing with control bread. Additionally, an improvement in nutritional value in terms of proteins (80%) and minerals content (P—50.0%, Mg—6.0%, and Ca—360.3%) was obtained, which can give an additional contribution to the nutritional daily requirements of celiac patients. Linear correlations between dough rheology properties and bread quality attributes were found, supporting the good breadmaking performance obtained.

Keywords: curd cheese enrichment; whey protein; mimic gluten structure; dough rheology; bread texture; gluten-free bread

1. Introduction

The demand for gluten-free products, especially bread, has been growing since the number of celiac disease diagnoses, as well as other gluten intolerance cases, have been increasing, requiring the absence of gluten from the diet [1].

Celiac disease is characterized by a small gut inflammation via autoimmune response to specific peptides of gliadin, one of the gluten proteins [2]. This gluten intolerance can lead to several health-associated disorders, as the damage and atrophy of the gut villi results in malabsorption of nutrients [2], with a great impact on health.

Gluten is the main structure-forming protein in flour, responsible for the dough viscous-elasticity features that contribute to a good appearance and crumb structure of the baked goods [3]. The protein fractions in the gluten network are glutenin and gliadin: the former gives elasticity to dough, while gliadin produces a viscous, fluid mass, under hydration conditions. The gluten matrix is a protein structure determinant of wheat dough properties such as dough resistance to stretch, extensibility, gas retention capacity, bread volume, and softness, responsible for the quality of final products. Therefore, gluten removal results in major technological problems leading to low quality and short shelf life of the gluten-free products [1]. The technological challenge of gluten removal has led to the search for alternatives to mimic gluten structure in the manufacture of gluten-free bakery goods [1–7].

In addition, the nutritive value of the gluten-free bread (GFB) presents, generally, a deficient nutritional profile of protein and minerals, but high contents of carbohydrates and fat [4].

To overcome this challenge, several approaches were taken in the last few years, as the use of gluten-free cereal flours (e.g., rice and corn flours) [5], pseudocereals (e.g., quinoa, amaranth, buckwheat) [6], and starches (corn, potato, cassava) [5–8]. To mimic the gluten-like structure, the usage of certain hydrocolloids such as hydroxypropyl-methyl-cellulose and guar and/or xanthan gum [5,7], have been widely tested to enhance the dough viscoelastic features and GFB technological properties [8].

Proteins are naturally good hydrocolloids and one of the main molecule classes available to improve desirable textural attributes, promoting crosslinking and aggregation mechanisms, with a great impact on food structure. They are extensively used in different gluten free formulations, such as egg albumin and whey protein [1,9], due to their functionality, based on water binding and holding capacity, emulsifier, foaming, and gelling properties. In addition, proteins present a key role in Maillard reactions, which are responsible for color development, needed to improve the appearance of GFB crust [10].

Dairy proteins are interesting ingredients to enhance the nutritional properties of GFB due to their protein content and balanced amino acids profile, minerals content as calcium and phosphorus, as well as functional properties on baking performance [11].

A recent work showed that the incorporation of fresh dairy products on wheat bread formulations increased the nutritional value and improved the texture and other bread quality attributes [12]. Other researchers showed relevant enhancements in breadmaking, by the addition of whey protein (WP) on GFB formulations, reporting the capacity to increase water absorption, improving the handling properties [13]. Good thermal gelation capacity of WP inducing viscoelastic gels by protein network, capable of supporting dough foaming, was also reported [14].

Curd cheese (Cc) is a coproduct obtained by the thermal denaturation and subsequent precipitation of the soluble whey proteins. These products are considered to have a high protein nutritional value, representing of 20–30% of the proteins present in bovine milk (beta-lactoglobulin, alfa-lactalbumin) and are considered to be an important source of essential amino acids (leucine, isoleucine, and valine) and minerals (e.g., Ca and P) [15].

Therefore, the enrichment of GFB with fresh Cc can be an interesting approach to enhance their nutritional value, and the combination between vegetable (cereal) and animal proteins (denatured whey protein from Cc) could represent an alternative to mimic a foam structure support like the gluten network, improving bread quality.

This research work aimed to evaluate the influence of the fresh Cc addition on gluten-free breadmaking performance, to improve the GFB quality. Various levels of Cc addition into a gluten-free dough were tested, and the effect on dough rheology properties, by mixing curves, steady shear flow behavior, and small amplitude oscillatory assays, was assessed. The impact on GFB quality, by evaluating the loaf firmness and staling kinetics during the storage time, as well as by the after-baking quality attributes and the nutritive value, were also evaluated.

2. Materials and Methods

2.1. Raw Materials

GFB were prepared using rice flour (Próvida, Pêro Pinheiro, Portugal), buckwheat flour (Próvida, Pêro Pinheiro, Portugal), and potato starch (Colmeia do Minho, Paio Pires, Portugal) according to the GFB recipes earlier described by Graça et al. [16]. Detailed nutritional composition of the gluten-free flours used were described earlier by other authors [16].

The fresh Cc lactose-free used was a commercial product from Lacticínios do Paiva (Lamego, Paiva, Portugal), obtained by the soluble protein thermal denaturation/precipitation of the whey resulting from the production of the lactose free cheeses; the dry extract of Cc (31.2%, dry matter) and respective nutritional composition were determined and described in previous works [12,16].

Other ingredients used were commercial saccharose (Sidul, Santa Iria de Azóia, Portugal), salt (Vatel, Alverca, Portugal), dry yeast (Fermipan, Setúbal, Portugal), vegetable fat (Vegê, Sovena Group, Algés, Portugal), and xantham gum (XG) (Naturefoods, Lisboa, Portugal) [12,16].

2.2. Gluten-Free Bread Dough Preparation

GFB dough formulations were prepared according to the procedure earlier described by Graça et al. [16]: the yeast was activated in warm water; dry ingredients were incorporated, well mixed, and kneaded for 10 min; dough was fermented/leavened for 20 min at 30 °C, followed by baking at 180 °C during 30 min under convection, according to the breadmaking conditions earlier described [16]. Baking trials were performed in triplicates.

The GFB formulations tested are presented in Table 1.

Table 1. Bread formulations tested: control bread (CB) and curd cheese breads (CcB).

Ingredients (% *w*/*w*)	CB	CcB5%	CcB10%	CcB15%	CcB20%
Buckwheat	16.6	14.6	12.6	10.6	9.6
Rice	25.0	22.0	20.0	16.6	13.0
Potato starch	14.0	12.0	11.0	10.4	8.0
Curd cheese	0.0	5.0	10.0	15.0	20.0
Water added **	37.0	35	32.0	31.0	30.0
Curd cheese water ***	0.0	4.0	7.0	9.0	12.0
Total water absorption ****	37.0	39.0	39.0	40.0	42.0

Other ingredients kept constant: 7.4%; ** Determined by MicrodoughLab mixing curves; *** Water deriving for curd cheese incorporation; **** Total water absorption = water added + water deriving from Cc incorporation.

Considering the water coming from the different Cc additions tested, the water added (WAD) was determined by MicrodoughLab mixing curves, and the water absorption was calculated according to the procedure earlier described by Graça et al. [16]. Cc doughs were prepared by successive incorporations of 10, 20, 30, and 40 g of Cc into dough (5% up to 20% *w*/*w*). Replacements were based on gluten-free flours basis, substituting the dry extract of each Cc percentage on 100 g of flour [16]. Ingredients kept constant: salt—0.8%; saccharose—1.6%; yeast—1.6%, XG—0.3%, and vegetable fat—3.1% (sum of ingredients = 7.4%) (Table 1).

2.3. MicrodoughLab Measurements

Mixing Curves

Mixing dough behavior of control dough and doughs produced with Cc additions, was studied using the MicrodoughLab equipment (Perten instruments, Hägersten, Sweden), according to the specific protocol: 4 g of gluten-free flours mixed for 20 min at 30 °C under constant speed, 63 rpm. The parameters recorded from the mixing curves that characterize the dough rheology behavior are (AACC, 54–60.01): (WA) water absorption, or the amount of water required to reach the optimal dough consistency (mL of water/100 g of flour, at 14.0% of moisture basis), (DDT) dough development time or required time to reach the maximum dough consistency, (DST) dough stability during mixing, corresponding the time at which dough kept the maximum consistency; (DSF) dough softening degree or mixing tolerance index. Mixing curves were triplicated.

2.4. Dough Rheology Characterization

The dough rheology measurements were assessed according to the method earlier described by Graça et al. [16]. Briefly, rheology measurements (Haake Mars III—Thermo Scientific, Karlsruhe, Germany, Universal Temperature Control-Peltier system), on the fermented dough (20 min), using a serrated parallel plate as sensor system (PP35 and 1 mm gap), to avoid the split effect [17], and under cold conditions (at 5 °C) to inactivate the yeast, were performed in triplicates.

2.4.1. Steady-Shear Dough Behavior

The influence of the Cc addition on dough viscosity behavior was evaluated according to the method earlier described by Graça et al. [16]: shear steady conditions, varying the shear rate varied from 1.00×10^{-6} to 1.00×10^{3} s^{-1}.

Experimental rheology data were compared and model by Carreau Model, applying Equation (1):

$$\eta = \eta_0/[1 + (\dot{\gamma}/\dot{\gamma}_c)^2]^{s} \quad (1)$$

where the fitted parameters obtained corresponds to: η is the apparent viscosity (Pa. s), $\dot{\gamma}$ is the shear rate (s^{-1}), η_0 is the zero-shear rate-viscosity (Pa. s), $\dot{\gamma}_c$ is the critical shear rate at the onset of the shear-thinning behavior (s^{-1}), i.e., the value corresponding to the transition from Newtonian to shear-thinning behavior and s is a dimensionless parameter related to the slope of this region.

2.4.2. Dynamic-Shear Dough Behavior

The impact of the Cc enrichment on dough linear viscoelastic properties, by the elastic (G’) and viscous (G”) moduli changes, by small-amplitude oscillatory measurements was applied, according to the method earlier described by other authors [16]: 0.001 to 100.0 Hz of frequency ranging, at 10 Pa of shear stress within the linear viscoelastic region (determined at 1 Hz).

In all rheology methods described above, triplicates were performed.

2.5. After-Baking Quality Parameters of the GFB

2.5.1. Bread Texture and Staling Kinetics

Bread crumb texture (BCT) was evaluated following the methods earlier described Graça et al. [12,16,18]: penetration mode, by puncture test, using a Texturometer Stable MicroSystems (Surrey, UK). Triplicates of each 10-bread crumb firmness measurement repetitions were performed.

BCT of the different Cc breads was compared in terms of firmness, and bread staling kinetics (during a storage time of 96 h), in accordance with a procedure previously described [12,16].

Applying the linear Equation (2) the bread staling kinetics was described as a function of time,

$$\text{Firmness} = A \times \text{time} + B \quad (2)$$

where A (N/h) corresponds to the staling kinetics and B (N) the initial firmness of the GFB [16].

2.5.2. Breadmaking Properties

Post-baking quality parameters of the GFB: moisture (%), water activity (aw), bake loss (BL), and specific bread volume (SBV) (cm^3/g), were evaluated, according to the procedures previously reported [12,16]. Triplicates were performed.

Bread crumb and crust color were recorded by Minolta colorimeter using illuminative D65 (Chromameter CR-400, Minolta-Japan), as earlier described [16].

Differences in bread color (ΔE) between control bread (CB) and curd cheese breads (CcB) was calculated by Equation (3):

$$\Delta E = [(L^* \text{ CcB} - L^* \text{ CB})^2 + (a^* \text{ CcB} - a^* \text{ CB})^2 + (b^* \text{ CcB} - b^* \text{ CB})^2]^{1/2} \quad (3)$$

2.6. Nutritional Value of the GFB

Nutritionally, the obtained GFBs were characterized based on the proteins (ISO-20483:2006), lipids (NP 4168), ash (AACC Method 08–01.01), carbohydrates (estimated by difference), and total minerals (ICP-AES-Inductively Coupled Plasma-Atomic Emission Spectrometry) contents, following the Standard Methods described previously by Graça et al. [12,16]. Triplicates were performed.

2.7. Statistical Analysis

Statistical analysis of the experimental data (average values and standard deviation) (RStudio, Version 1.1.423) using variance test in one factor (ANOVA), by Tukey test (post-hoc comparisons, at 95% of significant level), were assessed. A Nonlinear Rheology Model, to fit the experimental rheology data was applied (Carreau model using TA Instruments/TRIOS software).

3. Results and Discussion

3.1. MicrodoughLab Mixing Curves

The impact of Cc additions on dough mixing properties was evaluated and the mixing parameters, summarized in Table 2, were used to characterize the effect of Cc additions on mixing behavior.

Table 2. Comparison of Microdoughlab mixing parameters: WA—water absorption (%), DDT—dough development time (min), DST—dough stability (min), DSF—dough softening degree (mNm), for control dough (CD) and curd cheese doughs (CcD5% up to CcD20%).

Samples	WA (%)	DDT (min)	DST (min)	DSF (mNm)
CD	66.30 ± 0.60^{a}	0.80 ± 0.10^{a}	0.80 ± 0.10^{a}	3.30 ± 0.61^{a}
CcD5%	66.70 ± 0.60^{a}	0.70 ± 0.10^{a}	0.60 ± 0.20^{a}	3.00 ± 0.12^{a}
CcD10%	67.70 ± 0.50^{a}	0.70 ± 0.20^{a}	0.60 ± 0.10^{a}	3.00 ± 0.63^{a}
CcD15%	69.30 ± 0.50^{ab}	0.90 ± 0.50^{ab}	0.60 ± 0.15^{a}	5.30 ± 0.60^{b}
CcD20%	70.30 ± 0.60^{b}	1.00 ± 0.10^{b}	0.60 ± 0.10^{a}	6.00 ± 0.30^{b}

Different superscripts (^{a}, ^{b}) indicate significantly statistical differences between CB and CcB. (at $p \leq 0.05$, Tukey test).

From Table 2, it can be observed that up to 10% of incorporation, no significant ($p > 0.05$) impact on water absorption (WA), dough development time (DDT), dough stability (DST), and dough softening (DSF), was registered. However, for higher levels of Cc tested, 15% and 20%, the WA increased, probably due to the increased amounts of proteins in dough matrix, requiring more water to improve the connectivity of the network developed by starch-protein-xanthan gum, reinforced by hydrogen bonds [19]. The DDT also increased with 15% of Cc addition, resulting from the increase in solids content associated with difficulties in dough homogenization. In terms of dough stability, no significant differences were observed. However, Cc additions led to a significant ($p < 0.05$) increase of dough softening after 15% of Cc addition, reflecting the weakening of dough structure. Although the Cc addition promoted a slight impact on dough mixing properties, in terms of DDT and DSF, these effects can be considered as an additional support of denatured WP (coming from Cc) to dough plasticity or extensibility, which can be crucial during the fermentation process to dough expansion and gas retention.

3.2. Dough Rheology Characterization

3.2.1. Gluten-Free Dough Flow Behavior

Figure 1 shows the impact of Cc additions, on steady-shear flow behavior of gluten-free doughs, in comparison to control dough (CD).

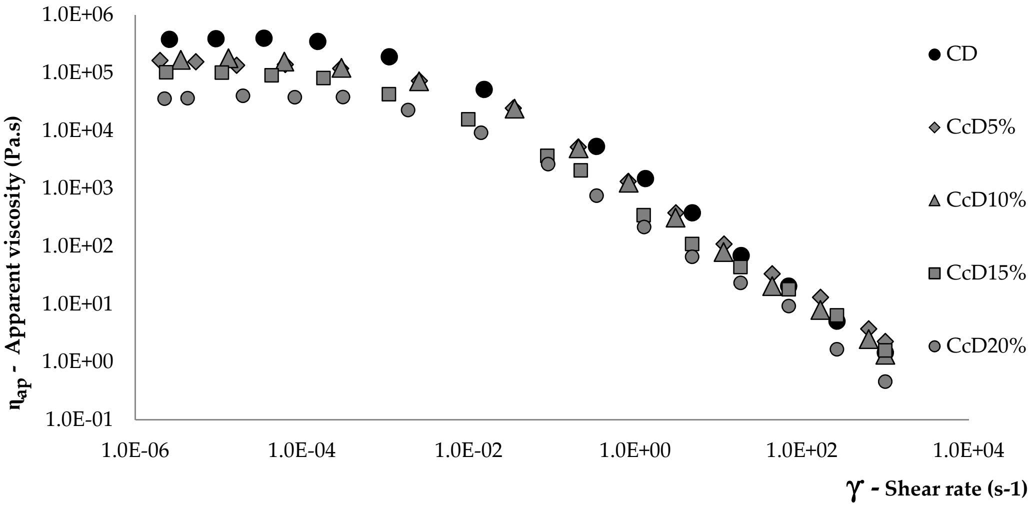

Figure 1. Flow rheology behavior of the control dough (CD) and Cc doughs (CcD): 5% up to 20%, *w*/*w*.

The flow curves indicated that all these systems behaved as typical structured materials, showing a Newtonian plateau region at low shear rates and dough viscosity began to decrease as the shear rate values increase, reflecting a typical shear-thinning behavior. These results agree with those obtained by other authors [19,20], using different gluten-free flours and hydrocolloids on GFB formulations.

Carreau equation was adjusted to the experimental data from Figure 1 ($R^2 > 0.975$) and the obtained rheology fitted parameters, summarized in Table 3, were used to characterize the dough flow behavior: Cc incorporations to dough promoted a significant reduction on apparent viscosity values (η_0) ranging from 390.2 k Pa s for CD to 37.3 k Pa s for CcD20% (higher Cc level tested), representing a decrease in viscosity of around 90.0%. Considering the significant reduction in dough viscosity observed, promoted by the Cc addition to dough, the dilution effect of starch-proteins-xanthan gum linkage density, of the GFB reference, should be invoked to support these findings.

Table 3. Fitted rheology parameters estimated for control dough (CD) and curd cheese doughs (CcD5% up to CcD20%), by applying Carreau Model**.**

	η_0 (k Pa s)	$\dot{\gamma}_c$ (s^{-1})	s (Slope)	R^2
CD	390.2 ± 8.7 ^{a}	$3.3 \times 10^{-3} \pm 1.3 \times 10^{-4}$ ^{a}	0.27 ± 0.06 ^{a}	0.987
CcD5%	166.4 ± 5.0 ^{b}	$3.1 \times 10^{-3} \pm 4.0 \times 10^{-4}$ ^{a}	0.26 ± 0.03 ^{a}	0.987
CcD10%	178.0 ± 6.6 ^{b}	$3.2 \times 10^{-3} \pm 2.3 \times 10^{-4}$ ^{a}	0.23 ± 0.03 ^{ab}	0.980
CcD15%	101.5 ± 1.7 ^{c}	$3.6 \times 10^{-3} \pm 4.8 \times 10^{-4}$ ^{a}	0.21 ± 0.04 ^{b}	0.996
CcD20%	37.3 ± 4.0 ^{d}	$3.6 \times 10^{-3} \pm 2.3 \times 10^{-4}$ ^{a}	0.18 ± 0.09 ^{b}	0.975

Different superscripts (^{a}, ^{b}, ^{c}, ^{d}) indicate significant statistical differences between CD and CcD (at $p \leq 0.05$, Tukey test).

Based on the values of critical shear rate ($\dot{\gamma}_c$), the transition of the Newtonian to shear-thinning behavior of all systems occurred around 3.4×10^{-3} s^{-1}.

Concerning the dough flow index (slope), the addition of Cc up to 10% did not significantly ($p > 0.05$) change the dough's flow behavior, presenting values like CD (0.27–0.23). Nevertheless, as the amount of Cc increases, the flow index was significantly reduced, varying from 0.27 (CD) to 0.18

for high Cc level tested (CcD20%). This increase in shear thinning behavior is probably due to the formation of longer and more flexible polymer-chains [21].

Similar findings were reported by other researchers [22] to evaluate the effect of other hydrocolloids addition on the rheology features of the GF cakes. According to a previous study [23], lower flow indexes led to a quick decrease in dough viscosity, which can improve the capacity of dough to expand and retain more gas during baking.

3.2.2. Gluten-Free Dough Viscoelastic Behavior

Rheology behavior of the GF doughs focused on storage (G′) and loss (G″) moduli changes with oscillatory frequency, was assessed on fermented doughs. Results are illustrated in Figure 2A–D, where different viscoelastic functions can be observed between CD and Cc doughs obtained.

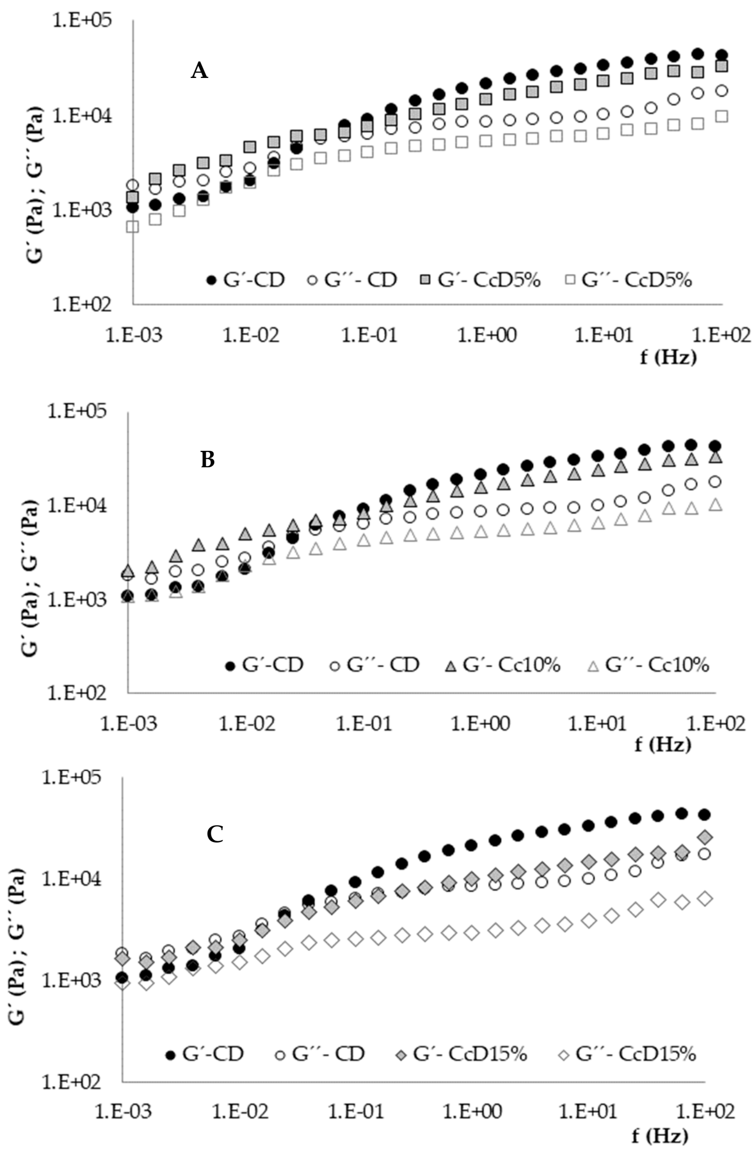

Figure 2. *Cont.*

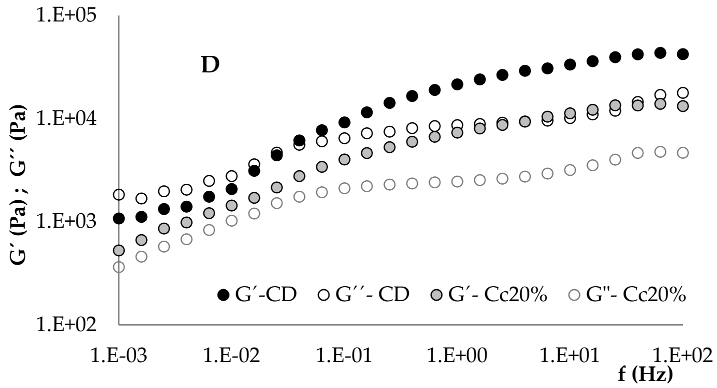

Figure 2. Rheology changes in viscoelastic functions, storage (G′, full symbols) and loss (G″, empty symbols) moduli, by the additions of curd cheese to dough (CcD5% up to CcD20% *w*/*w*), compared with viscoelastic profile of the control dough (CD) (black round symbols): (**A**) CD vs. Cc5%; (**B**) CB vs. Cc10%; (**C**) CB vs. Cc15%; (**D**) CB vs. Cc20%.

Starting for CD, at low frequency, a poorly structured system can be observed, since the values of G″ are higher than G′, characteristic of a typical viscoelastic fluid behavior [22]. Nevertheless, the crossover of both moduli occurred with the frequency increase, and the dominance of the G′ over the G″ was observed, expressing a weak gel behavior [24]. For Cc doughs, a typical weak-gel structure can be observed [25], where the G′ values were greater than the G″ over the whole range of frequency applied, indicating the predominance of the solid-elastic behavior [24], with high frequency dependence [26]. The values of both G′ and G″ were decreasing with Cc incorporations. The viscoelastic behaviors observed suggested that the presence of denatured WP, coming from Cc addition, are promoting a new network or/and protein agglomerates, giving more complexity to dough matrix. On the other hand, the increasing amount of lipids coming from the Cc additions enhance extensibility by the lubrication effect on the macromolecular chains resulting in the reduction of the viscoelastic parameters, increasing the plasticity of the doughs. The viscoelasticity of the gluten-free dough (GFD) depends strongly on intra and intermolecular interactions, i.e., a network with a desirable viscoelastic behavior, required for breadmaking. The chemical composition and functional properties of Cc proteins and their interactions are responsible for the changes in dough viscoelastic behavior.

From the results obtained, it can be stated that the Cc addition into dough promoted significant changes in dough viscoelastic profile, that probably can result in higher dough protein network ability to inflate and retain more CO_2, due to a flexible protein network, reducing considerably the dough viscoelastic parameters, under oscillatory measurements. Similar reports were obtained by other authors [27], where the addition of different protein sources to dough produced smaller G′ and G″ values, causing the reduction of consistency. This behavior can result in better quality properties based on the bread texture and volume [28].

3.3. *Quality Characterization of the Gluten-Free Bread*

3.3.1. Bread Crumb Texture and Hardening Kinetics

Bread crumb firmness is an important parameter used to evaluate the bread quality, that influences the consumers acceptability. To measure the effect of Cc addition on bread firmness, during 4 days of storage time (96 h), a puncture test was applied [12,16,18], to mimic the teeth chewing of the human sensory perception.

The mean values of bread crumb firmness (BCF) of CB and CcB are illustrated in Figure 3, showing that for all Cc amounts tested, bread crumb was softer than for CB, as the Cc levels increase in GFB formulations.

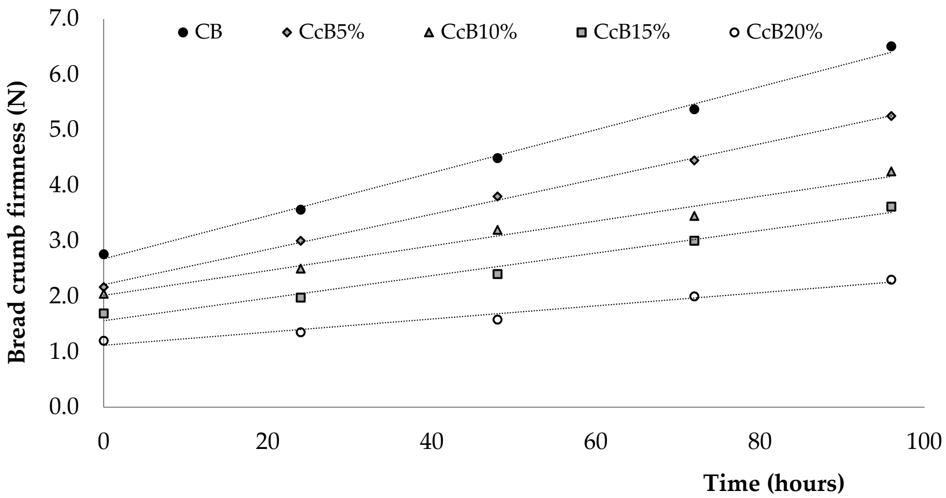

Figure 3. Evaluation of the gluten-free bread (GFB) crumb firmness progression, (96 h at room temperature), between control bread (CB) and curd cheese bread (CcB5% up to CcB20%).

Initial and final values of BCF varied from 2.70 N to 6.50 N for CB and 1.12 N to 2.30 N for CcB20%, representing a reduction on the firmness of around 55% (initial) and 65% (final). This behavior suggests a high functionality of the denatured WP in breadmaking performance. Higher firmness values of CB seem to be consistent with high viscosity and viscoelastic moduli values exhibited for CD, probably due to stiffer structure effect promoted by xanthan gum, reducing the dough expansion, consequently leading to firmer breads. Similarly, Demirkesen et al. [21] reported an increase of BCF by xanthan gum addition in GFB.

Staling of bread during the storage time is a consequence of humidity loss, and subsequent starch crystallization/retrogradation phenomena, leading to an increase of crumb firmness values [28].

Linear correlations were determined to describe the staling kinetics of the GFB, under controlled storage conditions (96 h at room temperature). The obtained linear parameters, summarized in Table 4, reflects the influence of Cc incorporations on the bread staling rate (Equation (2)).

Table 4. Linear parameters derived by linear correlation ($R^2 > 0.972$): A—bread staling rate (N/h) and B—initial bread firmness (N), for control bread (CB) and curd cheese bread obtained (CcB).

Cc Levels	Staling Rate (N/h)	Firmness (N)	R^2
CB	0.040 ± 0.001^{a}	2.70 ± 0.23^{a}	0.996
CcB5%	0.032 ± 0.002^{ab}	2.21 ± 0.40^{ab}	0.998
CcB10%	0.022 ± 0.001^{c}	1.90 ± 0.30^{b}	0.981
CcB15%	0.020 ± 0.003^{c}	1.57 ± 0.09^{c}	0.979
CcB20%	0.012 ± 0.002^{d}	1.12 ± 0.15^{d}	0.972

Different superscripts (^{a}, ^{b}, ^{c}, ^{d}) indicate significant statistical differences between CB and CcB: CcB5% up to CcB20% (at $p \leq 0.05$, Tukey test); A and B linear parameters were obtained by Equation (2): Firmness = A × time + B.

The Cc bread staling rate values were substantially lower than CB value, ranging from 0.032 N/h for lower incorporation (CcB5%) to 0.012 N/h for higher additions (CcB20%), compared to 0.040 N/h obtained for CD, a reduction about 70% of staling. Results reported by Gallagher et al. [13], by evaluating the effect of dairy powder addition on GFB shelf-life, are similar with those obtained. These findings are also in line with those obtained by other researchers [12], by testing the fresh dairy products addition to improve the wheat bread quality.

The functionality of the Cc protein should be considered to explain these results of bread texture and bread aging rate, whose positive effects can be associated to the capability of these proteins to bind with water and retain moisture, retarding the starch retrogradation and hence the increase of bread hardness [14]. On the other hand, these results suggest that the denatured WP relaxes the protein-starch-XG network, giving to dough a foaming support to retain the gases within, improving the bread texture by increasing volume (Figure 4) and softening effects [29]. Figure 4 shows the bread obtained with different levels of Cc addition, in comparison with CB.

Figure 4. Comparation of the resulted breads obtained for control bread (CB) and those breads obtained by different additions of curd cheese (CcB): CcB5% up to CcB20%.

3.3.2. After-Baking Parameters of the Gluten-Free Bread

Breadmaking performance of GFB produced with Cc additions was assessed by bread quality parameters: moistness (%) water activity (aw), baking loss (BL, %), specific bread volume (SBV, cm^3/g), crust and crumb color. Results are summarized in Table 5.

Table 5. Bread quality parameters: moisture, water activity (aw), baking loss (BL), specific bread volume (SBV), and bread crust and crumb color, for control bread (CB) and curd cheese breads (CcB5% up to CcB20%).

	CB	**CcB5%**	**CcB10%**	**CcB15%**	**CcB20%**
Moisture (%)	42.85 ± 0.33 ^{a}	43.02 ± 0.60 ^{a}	45.50 ± 0.43 ^{b}	47.80 ± 0.93 ^{c}	51.00 ± 0.13 ^{d}
aw	0.960 ± 0.006 ^{a}	0.951 ± 0.004 ^{ab}	0.943 ± 0.003 ^{b}	0.913 ± 0.005 ^{c}	0.892 ± 0.002 ^{c}
BL (%)	12.00 ± 0.40 ^{a}	11.00 ± 0.04 ^{ab}	10.22 ± 1.10 ^{b}	9.60 ± 0.80 ^{b}	9.00 ± 0.30 ^{b}
SBV (cm^3/g)	1.50 ± 0.11 ^{a}	1.90 ± 0.08 ^{a}	2.10 ± 0.11 ^{b}	2.30 ± 0.15 ^{bc}	2.50 ± 0.06 ^{c}
Crust color					
L*	59.60 ± 2.90 ^{a}	58.60 ± 1.64 ^{a}	51.80 ± 2.30 ^{b}	46.44 ± 3.13 ^{c}	36.80 ± 2.50 ^{d}
a*	8.40 ± 1.00 ^{a}	9.40 ± 0.51 ^{a}	10.10 ± 0.94 ^{b}	11.80 ± 0.80 ^{bc}	12.80 ± 0.30 ^{c}
b*	29.10 ± 1.43 ^{a}	29.10 ± 1.20 ^{a}	29.30 ± 1.10 ^{a}	21.20 ± 2.53 ^{b}	21.10 ± 2.40 ^{b}
ΔE		1.41 ± 0.90 ^{a}	8.00 ± 1.02 ^{b}	15.70 ± 1.80 ^{c}	24.60 ± 1.30 ^{d}
Crumb color					
L*	60.52 ± 2.90 ^{a}	63.81 ± 2.32 ^{a}	67.36 ± 1.90 ^{b}	69.59 ± 0.62 ^{b}	70.90 ± 1.91 ^{b}
a*	3.49 ± 1.05 ^{a}	5.49 ± 0.75 ^{a}	6.63 ± 0.89 ^{b}	7.83 ± 0.20 ^{c}	1.81 ± 0.23 ^{d}
b*	11.40 ± 0.20 ^{a}	11.10 ± 0.64 ^{a}	12.15 ± 1.30 ^{a}	18.53 ± 1.50 ^{b}	23.40 ± 1.10 ^{c}
ΔE		3.90 ± 0.95 ^{a}	7.60 ± 1.10 ^{b}	12.30 ± 1.20 ^{c}	16.00 ± 1.23 ^{c}

Different superscripts (^{a}, ^{b}, ^{c}, ^{d}) indicate significant statistical differences between CB and CcB: CcB5% up to CcB20% (at $p \leq 0.05$, Tukey test).

Moisture percentage of the breads ($p < 0.05$) increased by successively Cc additions, varying from 43.0% (CcB5%) to 51.0% (CcB20%), compared to 42.5% of CB, an increase of 21% in moisture. Water is the most important plasticizer in food structure, acting as chain polymer lubricant, reducing stiffer linkage forces by increasing flexibility of structure effects [30]. Water activity (aw) was slightly reduced as the Cc levels increase in bread formulations, from 0.960 for CB to 0.890 for CcB20% (upper Cc amount), a decrease of 7.3%, that will contribute to improving the preservation of bread during storage.

Additionally, a significant increase on yield for Cc bread was obtained, around 25% compared to CB, as the consequence of less weight loss during baking (BL). Improvement in water holding capacity has been previously reported in GFB systems, by protein sources addition, enhancing the bread yield after baking [27].

The enrichment of the bread formulation by Cc proteins addition enhanced the appearance of the breads, in terms of specific bread volume (SBV), from 1.50 cm^3/g for CB to 2.60 cm^3/g for CcB20%, a considerable increase about of 73%. These improvements in SBV could be justified by the increase in water absorption, improving the handling properties and subsequently led to a flexible starch-protein matrix further enhanced by starch gelatinization during baking [13].

The findings also agree with those reported by Sahagún and Gómez [27] evaluating the effect of other protein sources on physicochemical and quality properties of GFB.

Other important features of the GFB are the crust and crumb color, also highly associated with bread consumers acceptance. Crust and crumb color results (Table 5) reveal that the addition of Cc had a considerable impact on both quality bread attributes. The inclusion of Cc substantially ($p < 0.05$) increased the darker crust color of the GFBs (lower L* values). In fact, it could be related to the increments in protein levels by Cc additions, since the Maillard reaction occurs between amino acids and reducing sugars [31]. Based on bread crust tone, Cc addition promoted an increase of redness (higher a* values) reducing slightly the yellowness of the crust (lower b*). The differences in color (ΔE) between Cc breads and CB, were calculated by Equation (3), resulting in ΔE values higher than 5, indicating significant bread color differences by the increments in Cc. In agreement, are previous reports about GFB with egg white and whey proteins addition [1]. In contrast, on crumb color, lighter breads (L* values) with a remarkable yellowness tone (b* values) whilst the reddish tone decreased (a* values), was obtained. Differences in color were also confirmed by the values ΔE higher than 5. Similar results were previous reported Graça et al. [16] by adding yoghurt to improve the GFB quality properties.

3.4. The Relationship between Bread Quality and Dough Rheology Features

This study showed that the bread volume and crumb texture can be improved by denatured WP coming from Cc incorporation to dough, acting as lubricant agents in dough matrix. In fact, results discussed above suggest some linear correlation between bread quality attributes and dough rheology results, that can be taken into consideration to support the enhancements of the GFB.

Linear relationships ($R^2 > 0.900$) were found between bread firmness (BF) with dough viscosity (DV) and elastic modulus (G', at 1 Hz), and bread volume (SBV) with both DV and G', at 1 Hz, as can be observed from Figure 5A,B.

Improvements of BF and SBV were obtained for Cc doughs with lower viscosity and elasticity values, resulting in softer breads with better volumes.

It can be stated that the Cc incorporation to dough had a significant impact on GFB quality attributes, and such effects are probably due to additional support of the denatured WP to flexible effects on macromolecule interactions, improving the dough matrix performance to produce higher quality breads than CB.

These finding are aligned with those published by Graça et al. [16], reporting positive effects of the casein protein, present in yoghurt, on GFB quality properties.

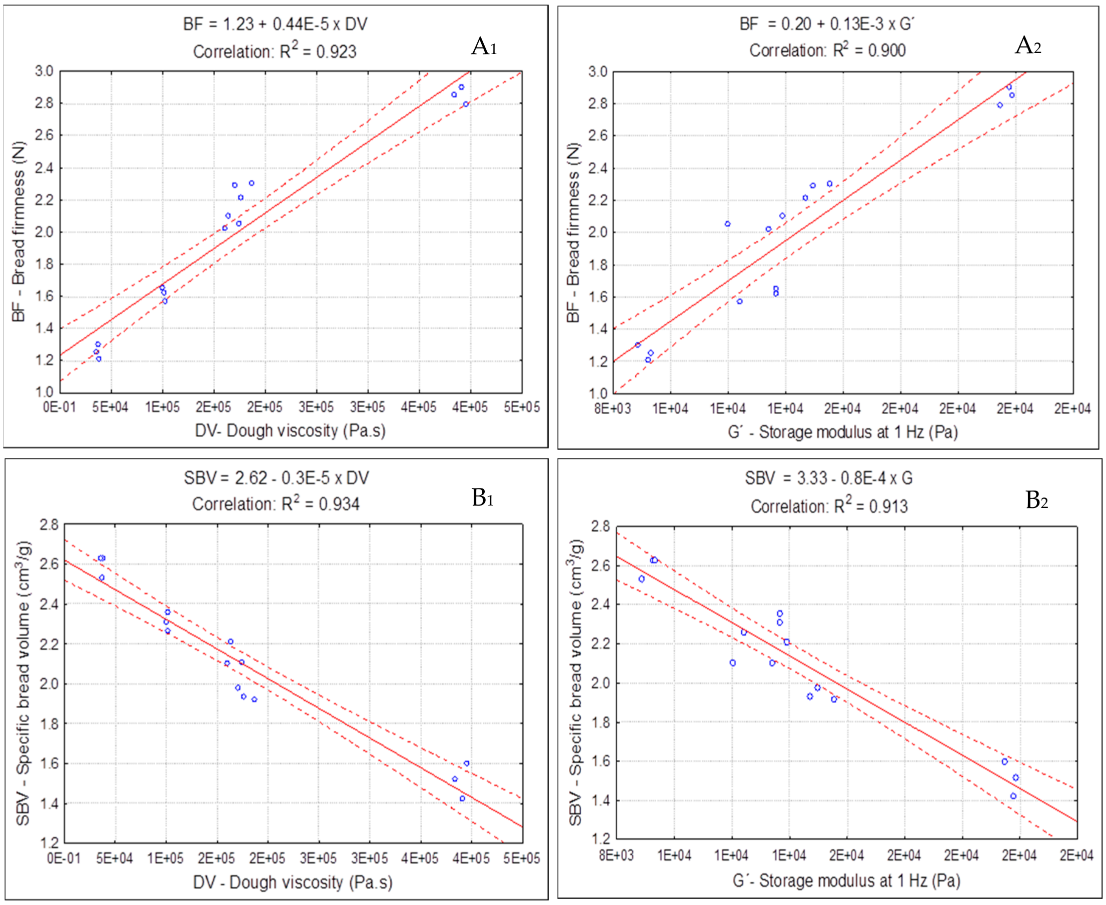

Figure 5. Linear relationship between bread quality attributes (BF—bread firmness and SBV—specific bread volume) with dough rheology properties (DV—dough viscosity and G′—elastic modulus, at 1 Hz): (**A$_1$**)—BF vs. DV; (**A$_2$**)—BF vs. G′; (**B$_1$**)—SBV vs. DV; (**B$_2$**)—SBV vs. G′.

3.5. Nutritional Value of the Gluten-Free Bread

The impact of Cc addition to improve the nutritional and minerals profile of GFB was evaluated, and the results obtained are summarized in Table 6.

The increments of protein by Cc incorporation to GFB was noticeable: comparing CB to CcB20% (higher level tested), the protein content varied from 5.34 to 9.40 g/100 g, respectively, representing an increase around 80%. Carbohydrates levels varied from 45.9 to 31.5 g/100 g, representing a considerable reduction of 31.4% for CcB20%, compared to CB. According to a recent work [32], in which the supplementation of wheat bread with curd cheese to reduce the glycemic response was performed, these results can suggest that the incorporation of Cc as baking ingredients in GFB formulations can be an alternative to obtain breads with reduced glycemic index.

In terms of lipids content, a significant increase of 35% for upper levels of Cc tested, was obtained.

A considerable increase in ash content was also observed, and this result is clearly reflected in terms of major minerals profile. As it can be observed from Table 6, a significant increase in phosphorus (50.0%), magnesium (6.0%), and calcium (360.3%) were registered for Cc bread, that can cover more than 15% of recommended daily value as stated in European community (CE) Regulation N° 1924/2006 based on nutrition and health claims made on foods [33], and in the Regulation (UE) N° 432/2012 that specifies a list of permitted health claims made on foods [34].

In terms of trace elements (Fe, Cu, Mn, and Zn), since their prevalence in buckwheat flour is higher than in curd cheese, their proportions were diluted by successive curd cheese additions.

A balanced gluten-free diet should provide adequate levels of macro- and micronutrients, since the diagnosis of celiac patients is often associated to deficiencies in protein levels and mineral components, caused by the atrophy and subsequent damage of small gut villus [35–37].

These nutritional improvements obtained by the addition of Cc on GFB formulation can give an additional contribution to fulfill the nutritional daily diet requirements in terms of protein and major mineral profile of celiac patients and also gluten-sensitive individuals.

Table 6. Nutritional value (g/100 g), including mineral profile (mg/100 g), for control bread (CB) and Cc breads (CcB5% up to CcB20%).

Nutrients (g/100 g)	CB	CcB10%	CcB20%	
Proteins	5.34 ± 0.21 ^{a}	7.60 ± 0.12 ^{b}	9.40 ± 0.10 ^{c}	
Lipids	4.30 ± 0.29 ^{a}	5.30 ± 0.61 ^{b}	5.80 ± 0.20 ^{c}	
Ash	1.40 ± 0.03 ^{a}	2.03 ± 0.11 ^{b}	2.90 ± 0.07 ^{c}	
Carbohydrates	45.90 ± 0.95 ^{a}	39.60 ± 1.50 ^{b}	31.50 ± 1.20 ^{c}	
Kcal	245.80 ± 1.80 ^{a}	236.20 ± 1.10 ^{a}	213.40 ± 2.10 ^{b}	
	Minerals (mg/100 g)			15% RDV * (mg/100 g)
Na (g/100 g)	0.41 ± 0.03 ^{a}	0.35 ± 0.01 ^{a}	0.36 ± 0.02 ^{b}	-
K	412.0 ± 8.02 ^{a}	315.35 ± 5.92 ^{b}	290.35 ± 10.40 ^{b}	300.0
P	131.80 ± 5.40 ^{a}	177.00 ± 0.90 ^{a}	197.40 ± 2.00 ^{b}	120.0
Mg	50.24 ± 1.02 ^{a}	50.60 ± 0.56 ^{a}	53.20 ± 0.82 ^{b}	45.0
Ca	35.93 ± 2.06 ^{a}	151.00 ± 0.81 ^{b}	165.38 ± 2.60 ^{c}	120.0
Fe	6.94 ± 0.12 ^{a}	3.68 ± 0.67 ^{b}	3.50 ± 0.05 ^{c}	2.1
Cu	0.23 ± 0.01 ^{a}	0.10 ± 0.02 ^{b}	0.07 ± 0.03 ^{c}	0.2
Mn	0.51 ± 0.01 ^{a}	0.40 ± 0.02 ^{a}	0.32 ± 0.03 ^{b}	0.3
Zn	1.12 ± 0.01 ^{a}	0.70 ± 0.08 ^{b}	0.65 ± 0.01 ^{b}	2.3

Different superscripts (^{a}, ^{b}, ^{c}) indicate significant statistical differences between CB and CcB: CcB5% up to CcB20%, (at $p \leq 0.05$, Tukey test). * Recommended daily values (RDV) established by Reg. (CE), No. 1924/2006 and Reg. (EU) No. 432/2012.

4. Conclusions

The findings from this work showed that the quality of the GFB can be substantially improved by Cc supplementation, and such effects are probably associated to the functionality of denatured WP and lipids from the Cc, enhancing the viscoelastic properties of the GFD, giving additional support to dough foaming structure.

The weakening effects observed on dough rheology features, promoted by Cc lipids incorporation led to a significant improvement in bread softness with higher specific volumes than CB. Linear correlation between dough viscosity and storage modulus, with bread firmness and specific volume, supported these findings, showing that the positive effects observed are proportional to the increased levels of the curd cheese into the gluten-free dough.

Nutritionally, a considerable protein enrichment as well as major minerals profile, especially calcium, phosphorus, and magnesium contents, were obtained, whereas the carbohydrates amount was significantly reduced.

Considerable improvements in terms of bread quality of the gluten-free bread were achieved by successive increments of curd cheese, especially on the higher levels tested (15% and 20% *w*/*w*).

In summary, it can be stated that the breadmaking performance, technology properties, quality attributes, as well as the nutritive value of the gluten-free bread, can be substantially improved by fresh Cc enrichment.

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