

## Research Article

# The Effects of Ovine Whey Powders on Durum Wheat-Based Doughs

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Two types of ovine whey powder, with different protein content, were added at increasing substitution rates to two types of semolina, one with strong and tenacious gluten and the other with weak and sticky gluten. For each dough the optimum mixing time and hydration level were calculated using the consistograph. The whey powder negatively affected the leavening volume of all doughs, at all percentages except the lowest one (5%), mainly because of its effects on the elastic component of gluten as measured with a stress relaxation test. Differences of the secondary structure of gluten proteins among samples were investigated by analyzing the amide I band in the Fourier transform infrared spectra of the dough. Weak and strong semolina showed a different relative percentage of  $\alpha$ -helix, random coil, and  $\beta$ -sheet structures. The longer mixing times for dough formation when using semolina with strong gluten led to an increase in  $\alpha$ -helices and random coils, which caused a worse leavening performance than the weak-gluten semolina.

## 1. Introduction

Semolina from durum wheat is used to make different types of local breads in the Mediterranean area, particularly in Southern Italy [1]. The chemical and rheological characteristics of semolina greatly affect the handling properties of the dough, its leavening capacity, and the final quality of the bread. Semolina characterized by a strong gluten network, resulting in high gluten index and  $P$  to  $L$  values, is suitable for producing soft bread with low specific weight [2] due to the high leavening and gas retention capacity. In contrast, semolina with a weak-gluten network results in dough that is not able to expand well, thus producing bread that is heavy and hard with an inhomogeneous structure [3].

In recent years, an increasing consumer desire for high food quality has led to renewed interest in ancient raw materials and traditional food production [4]. The importance of this topic was stated at the Universal Exhibition hosted in Milan in 2015 [5] where “the best of the agri-food and gastronomic traditions of each of the exhibitor countries”

were presented. In this context, local landraces and old wheat varieties have recently been rediscovered, and there has been a general effort to reintroduce them into the bakery industry. However, the replacement process has been hindered by their poor agronomic characteristics and poor gluten quality, which renders them unsuitable for bread making in modern bakery plants.

The functional properties of dough can be improved by the addition of additives as whey powder, which is commonly used as a food fortifier or as a source of low-cost proteins [6]. Whey lactose and proteins can be added to obtain food texture modifications (gelling, film-forming, foaming, and emulsifying) [7, 8]. Whey proteins also have important nutritional and biological functions, since the protein components and their peptide fragments are bioactive; for example, they exert antimicrobial and antiviral actions as well as anticarcinogenic activity and they have the capacity to modulate the innate immune system [9]. Moreover, 1 million tons of ovine whey is produced annually in Southern Italy [10], resulting in a number of environmental problems that include

pollution. Currently, the conversion of by-products from the dairy industry into dry products is one of the main industrial conversion processes. A number of scientific papers have investigated the use of whey powders derived from bovine milk, whereas fewer scientific reports are available on ovine whey powder [11].

The purpose of this study was to investigate the effect of adding ovine whey powder (OWP) in semolina-based doughs. Two types of low-grade semolina were used to prepare the doughs: one derived from a blend of strong durum wheat cultivars; the other was obtained from *Senatore Cappelli* cultivar, an old and tall durum wheat genotype, characterized by weak and sticky gluten, that poorly fits with the technological requirements of modern industrial plants. The effects of increasing substitution rates of OWP on the two types of dough were investigated. The physical-chemical properties and rheological behaviour of the doughs were evaluated and leavening trials were conducted. Potential molecular modifications in gluten conformation caused by the addition of OWP were investigated using Fourier transform infrared (FT-IR) spectroscopy.

## 2. Materials and Methods

**2.1. Raw Materials, Dough Preparation, and Analyses.** Two types of commercial low-grade semolina were purchased from a local mill (Molino Galleu, Sardinia, Italy). The first, referred to as 4T, was obtained by milling the *Senatore Cappelli* cultivar, and the other, referred to as 48T, derived from a blend of durum wheat cultivars that are commonly used to make pasta and semolina-based breads. Two types of commercial OWP (Alimenta Srl, Sardinia, Italy), referred to as A and B, were added to the dough formulations at different substitution rates (0, 5, 10, and 15% w/w). Hereafter, dough samples will be denoted by the type of semolina (4T or 48T) and the OWP type (A or B) followed by the relative percentage (e.g., 4TA10 means semolina 4T added with OWP A at the percentage of 10%). Moisture (%), ash (%), and protein content (%) calculated on a dry basis (d.b.), gluten index (%), and dry gluten content (% d.b.) were measured on semolina following the AACC Approved Methods 44-15A, 08-12, 46-12, 66-20, 38-12A, and 54-30A, respectively [12]. The latter method was adapted to durum wheat according to Dubois et al. [13]. The Alveo-Consistograph (Chopin Technologies, France) was used to determine water adsorption capacity at a consistency of 2,200 mbar (fixed moisture basis of 15%), the pressure drop after 450 s of mixing (D450, mbar), and the time to reach the target consistency of 2,200 mbar (TPrMAX), according to the AACC Approved Method 54-50 [12]. The dough was prepared in the consistograph mixer bowl at a fixed temperature (24°C) and at adapted hydration. OWP at different percentages, semolina, and yeast (1% w/w, total weight of semolina plus OWP A or OWP B) were mixed in the consistograph mixing bowl for 2 min before then addition of 2.5% saline solution. The mixing process was interrupted when the value of the dough consistency was 75% of the maximum pressure, after having exceeded the maximum pressure (2,200 ± 7% mbar) (Figure 1), as for Vinci et al. [14].

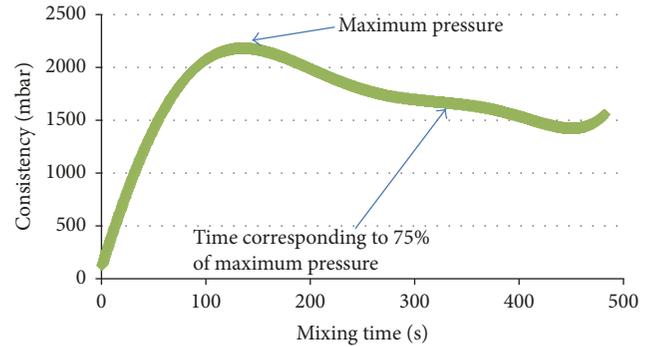


FIGURE 1: Curve from the consistograph: dough consistency versus mixing time. The mixing time chosen in this work is indicated.

After mixing, the dough was sheeted and formed for alveographic analysis. Another dough was prepared for each treatment, and when the desired consistency was reached, it was extruded, sheeted, and divided into five pieces that were used for a stress relaxation test with a texture analyzer. A third dough for each treatment was prepared in the same way in triplicate and used for the leavening trial.

**2.2. Stress Relaxation Test.** The TA.XT2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) equipped with a 30 kg load cell and a P50 probe was used for the stress relaxation test on the dough [15] and force (N) versus time (s) curves were recorded using Texture Expert Exceed software, version 2.64. The test speed was 1 mm s<sup>-1</sup>. The instantaneous strain applied to the dough was 10%, and the resulting stress was recorded for 40 s. A generalized Maxwell model was used to fit the stress relaxation data in accordance with Campus et al. [16], as follows:

$$\sigma(t) = \sum_{i=1}^n C_i \left( e^{-t/\tau_i} \right) + \sigma_e, \quad (1)$$

where  $\sigma$  is the stress (N) at a given time,  $t$  (s),  $C_i$  is the stress relaxation constant (N),  $\sigma_e$  is the equilibrium stress (N), and  $\tau_i$  (s) are the relaxation times of the Maxwell elements. TableCurve 2D version 5.01 (Systat Software Inc., San Jose, CA, USA) was used to perform regression analyses on the stress relaxation data using the Levenberg-Marquardt method.  $R^2$  (i.e., the percentage of explained variation) and maximum relative difference (MRD) were used to evaluate the goodness of fit. Only the relaxation part of the curve was used for the fitting procedure. A model with three Maxwell elements ( $n = 3$ ) was optimal to describe the viscoelastic behaviour of the dough ( $R^2 \leq 0.98$  and  $\text{MRD} \leq 7$ ). The maximum force achieved in the stress relaxation test was  $3.8 \pm 0.3$ , and it was the same in all the doughs, confirming that homogeneous dough was obtained in terms of consistency.

**2.3. Leavening Trial.** Leavening tests were performed as described by Vinci et al. [14]. For each treatment, the dough was prepared in the consistograph mixing bowl, as described in Section 2.1. After the preparation of the dough, 100 g was

TABLE 1: Properties of low-grade semolina (4T, 48T) and ovine whey powders (A, B).

Batch	Ashes (% d.b.*)	Protein content (% d.b.)	Moisture (%)	Gluten index (%)	$P^{**}$ (mm)	$L^{**}$ (mm)	$W^{**}$ ( $J * 10^{-4}$ )	$P/L^{**}$
4T	1.02 ± 0.02	11.1 ± 0.09	15.4 ± 0.10	56.0 ± 0.01	59.9 ± 1.8	49.0 ± 6.9	91.9 ± 6.4	1.24 ± 0.20
48T	1.03 ± 0.02	12.9 ± 0.48	15.2 ± 0.05	92.2 ± 0.02	98.2 ± 2.1	45.8 ± 5.7	173.7 ± 8.9	2.18 ± 0.29
A	8.50 ± 0.05	15.1 ± 0.15	4.1 ± 0.12	-	-	-	-	-
B	6.00 ± 0.04	35.2 ± 0.18	4.2 ± 0.08	-	-	-	-	-

\* Dry basis. \*\* Alveograph parameters at constant hydration:  $P$ , maximum overpressure;  $L$ , index of swelling;  $W$ , deformation energy;  $P/L$ , configuration ratio. Data were expressed as mean ± standard deviation.

transferred to graduated glass cylinders (250 mL capacity and 15 mm diameter) and left at 25°C until maximum leavening volume was attained. The increase in the height of the dough was recorded every 15 min.

**2.4. FT-IR Spectroscopy.** FT-IR spectral measurements were performed under vacuum conditions using the Bruker infrared Vertex 70 interferometer (Bruker Optik, Ettlingen, Germany) equipped with a deuterated triglycine sulfate detector, and spectral data were processed using Bruker OPUS 6.5 software. The dough samples were freeze-dried before FT-IR analysis to avoid interference from water [17]. After freeze drying, each dough sample was homogenized using an agate mortar and pestle, after which an aliquot of 3 mg was mixed with 297 mg anhydrous potassium bromide (KBr, >99%; Sigma-Aldrich, St. Louis, MO, USA) and pelleted for the analyses. The spectra were recorded in the 400–4000  $\text{cm}^{-1}$  range by averaging 128 scans at a resolution of 4  $\text{cm}^{-1}$ . The background was evaluated by measuring the KBr signals. Three replicates were measured for each treatment. The amide I band was studied in the spectral range from 1710 to 1580  $\text{cm}^{-1}$ . After normalization, A and B spectra were subtracted from the spectrum for the dough, after being multiplied by their relative percentage in the dough. The peak positions, corresponding to the secondary structure of the proteins, were identified using the second derivative spectra and used to initialize the best fit of the amide I band in the abovementioned spectral range. The best fit was obtained using the mixed Gaussian-Lorentzian function. The percentages of integrated peak areas were then calculated and referred to the secondary structure of protein, following the assignments of Wang et al. [18].

**2.5. Statistical Analysis.** Principal component analysis (PCA) was performed on the correlation matrix of the variables related to semolina and dough properties, mixing parameters, and volume of leavening. The component loadings were calculated as simple correlations (using Pearson's  $r$ ) between the components (i.e., the component scores for each dough) and the original variables. Data related to properties of dough (mixing time, volume of leavening, and protein content) were analyzed by one-way analysis of variance (ANOVA). When appropriate, mean separation was performed according to Duncan's multiple range test at  $p \leq 0.05$ .

### 3. Results and Discussion

**3.1. Characteristics of Semolina and OWP.** The chemical and physical properties of the two types of semolina and OWPs are shown in Table 1. The data show the differences between the two semolina samples with regard to protein content, gluten index, and the alveograph parameters configuration ratio ( $P/L$ ) and deformation energy ( $W$ ). 48T had a higher protein content, gluten tenacity, and extensibility than 4T; the latter was obtained from a wheat cultivar that is well known for its poor gluten quality, due to the pattern of the high molecular weight glutenin subunit 20 (HMW-GS 20), which is the predominant pattern in durum wheat landraces and old genotypes [19]; however, in new cultivars, as those of 48T, it has been almost completely replaced by the 6 + 8 and 7 + 8 HMW patterns, which exhibit stronger dough properties and superior baking quality than HMW-GS 20 cultivars.

There were differences in the chemical composition of the two OWPs, with B having a higher protein content and lower ash content than A (Table 1).

**3.2. Effect of OWPs on Rheological and Leavening Properties of the Dough.** Rheological, physical, and chemical data, collected from 4T and 48T doughs supplemented with OWP, were correlated and processed using PCA. The results of the PCA analysis are reported in Figure 2 and Table 2. The first two PC axes explained 85.3% of the differences among samples (Figure 2). Almost all of the variables contributed, both positively and negatively, to the first axes, whereas the original variable that contributed most to the second axis was the relaxation time of the Maxwell model  $\tau_i$ . The position of the samples with respect to the first PC axis was consistent with the addition of whey proteins, which moved the samples to the negative part of the first principal component (PC1). The negative association between PC1 and the protein content of the dough ( $r = -0.90$ ), and the positive association between PC1 and the volume of leavening ( $r = 0.92$ ), highlights the negative effect of whey addition on the leavening volume of semolina (Table 2). Commonly the leavening capacity is positively related to the protein content of the dough [20]; however, in this case, although the addition of whey increased the protein content, it also greatly strengthened the dough and probably led to greater resistance to extension. The correlations among the original variables confirmed this hypothesis, as strong

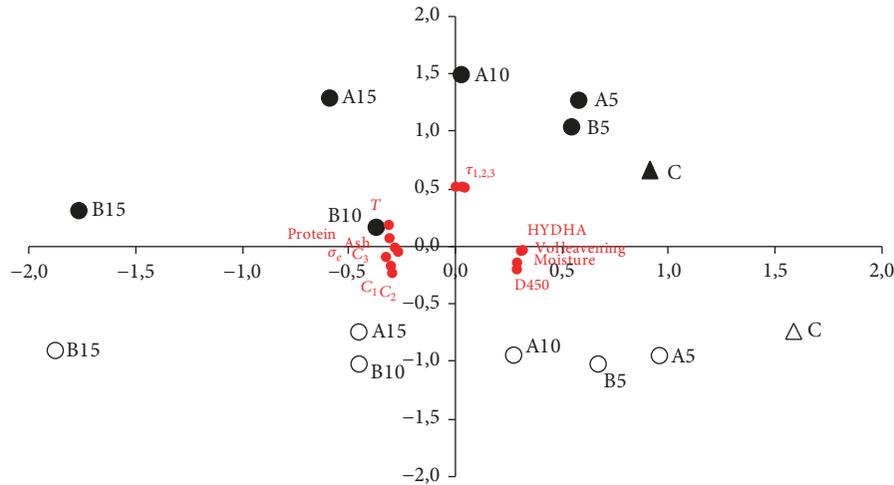


FIGURE 2: Principal component analysis (based on the correlation matrix) of the dough samples. Biplot of factor scores (points). 4T control ( $\Delta$ ); 48T control ( $\blacktriangle$ ); 48T dough with ovine whey powder (A, B) ( $\circ$ ); 48T dough with ovine whey powder (A, B) (black circle); loadings (red circle). Stress relaxation test parameters:  $C_1$ ,  $C_2$ , and  $C_3$ : decay force of the Maxwell model;  $\tau_1$ ,  $\tau_2$ , and  $\tau_3$ : relaxation times of the Maxwell model;  $\sigma_e$ : the residual modulus that remained unrelaxed. Alveograph parameters at adapted hydration:  $T$ : maximum overpressure (mm). Consistograph parameters: HYDHA: actual hydration of dough used in the test (15% mb); D450 dough weakness at 450 s. Vol leavening: leavening volume (mL).

TABLE 2: Proportion of total variation and eigenvectors of principal component (PC) axes in a principal component analysis performed on all the traits measured.

	Eigenvectors		Loadings	
	Axis 1*	Axis 2**	Axis 1	Axis 2
Moisture	0.29	-0.14	0.84	-0.25
Ash	-0.29	-0.01	-0.83	-0.01
Protein	-0.31	0.08	-0.90	0.14
HYDHA	0.31	-0.03	0.89	-0.06
D450	0.29	-0.19	0.84	-0.36
$T$	-0.31	0.19	-0.91	0.36
Vol leavening	0.32	-0.03	0.92	-0.05
$C_1$	-0.30	-0.16	-0.89	-0.30
$\tau_1$	0.04	0.52	0.12	0.96
$C_2$	-0.30	-0.23	-0.87	-0.42
$\tau_2$	0.03	0.52	0.10	0.98
$C_3$	-0.33	-0.09	-0.95	-0.16
$\tau_3$	0.00	0.52	0.01	0.98
$\sigma_e$	-0.27	-0.04	-0.78	-0.08

Component loadings are the simple correlations between the principal components and the original variables. \*Proportion of total variation is 60.3%.

\*\*Proportion of total variation is 25.0%. Stress relaxation test parameters:  $C_1$ ,  $C_2$ , and  $C_3$ , decay force of Maxwell model;  $\tau_1$ ,  $\tau_2$ , and  $\tau_3$ , relaxation times of the Maxwell model;  $\sigma_e$ , the residual modulus that remained unrelaxed. Alveograph parameter at adapted hydration:  $T$ , maximum overpressure. Consistograph parameters: HYDHA, actual hydration of dough used in the test; D450, dough weakness at 450 sec. Vol leavening, leavening volume.

associations among protein content and all of the indices of elasticity and strength of the dough (i.e., alveographic tenacity of the dough  $T$ , Maxwell decay force  $C_i$ , and equilibrium stress  $\sigma_e$ ) were found. The strong correlations between the variables and PC1 established a strong relationship among

them, notably between the leavening capacity of the dough and the consistograph parameters. Here, data from the consistograph (Table 3) show that increasing amounts of OWP lead to reduced water adsorption of the dough (HYDHA), in agreement with other authors [21, 22]. Note that the available literature refers to flour from *Triticum aestivum L.*, and no data have been published to date on the effects of the addition of whey protein to semolina-based dough. The effect of OWP on the dough was revealed by the higher TPrMAX and mixing time values (Table 3) and the concomitant lowering of D450 values (Table 3), which indicated an increase of dough strength and dough stability as the OWP percentage increased, similarly to the findings reported by Madenci and Bilgiçli [23].

In a previous study [14], we highlighted the key role of the technological parameters (i.e., hydration level of the dough and mixing time) in influencing the leavening volume of a semolina-based dough. We suggested that the optimum mixing time approximately corresponded to the time required to reduce the maximum pressure to 75% of its value (Figure 1), which resulted in a softer and more elastic dough that was better suited to leavening. This procedure was applied in the current study to make sure that all dough samples were mixed until the best consistency for leavening was attained, which explains why the mixing times were extremely variable among the samples, ranging from 330 s to 1,065 s (Table 3). As expected, the shortest mixing time was in the 4TC. The dough made from the stronger 48T showed a longer mixing time than the dough made from 4T in all treatments. We generally observed that the addition of OWP led to an increase in mixing time, consistent with the degree of substitution of semolina with OWP (Table 3). The leavening volume of 4TC was higher than that of the 48T samples (Table 3), although 4TC had a lower protein content and a poorer gluten quality,

TABLE 3: Properties of dough samples. TPrMax, HYDHA, mixing time, and D450 are Consistograph parameters.

Samples	TPrMAX (s)	HYDHA (%)	Mixing time (s)	D450 (mb)	Volume leavening (mL)	Protein content (%)
4TC	110.5 <sup>g</sup>	50.3 <sup>a</sup>	330 <sup>g</sup>	880 <sup>a</sup>	155 <sup>a</sup>	11.07 <sup>l</sup>
4TA5	147.5 <sup>fg</sup>	45.0 <sup>cd</sup>	480 <sup>e</sup>	528 <sup>b</sup>	165 <sup>a</sup>	11.65 <sup>i</sup>
4TA10	234.5 <sup>df</sup>	39.9 <sup>f</sup>	697 <sup>d</sup>	357 <sup>c</sup>	139 <sup>b</sup>	12.10 <sup>hi</sup>
4TA15	325.0 <sup>ad</sup>	36.8 <sup>g</sup>	930 <sup>b</sup>	211 <sup>ef</sup>	115 <sup>de</sup>	12.72 <sup>g</sup>
4TB5	150.5 <sup>fg</sup>	45.5 <sup>c</sup>	480 <sup>e</sup>	604 <sup>b</sup>	142 <sup>b</sup>	12.70 <sup>g</sup>
4TB10	243.0 <sup>cf</sup>	41.0 <sup>e</sup>	765 <sup>c</sup>	364 <sup>c</sup>	86 <sup>f</sup>	15.08 <sup>cd</sup>
4TB15	385.0 <sup>ab</sup>	35.9 <sup>g</sup>	892 <sup>b</sup>	119 <sup>fg</sup>	67 <sup>g</sup>	15.54 <sup>b</sup>
48TC	150.0 <sup>fg</sup>	48.5 <sup>b</sup>	420 <sup>f</sup>	585 <sup>b</sup>	135 <sup>bc</sup>	12.86 <sup>g</sup>
48TA5	218.5 <sup>ef</sup>	44.2 <sup>d</sup>	682 <sup>d</sup>	327 <sup>cd</sup>	135 <sup>bc</sup>	12.53 <sup>gh</sup>
48TA10	398.0 <sup>a</sup>	39.3 <sup>f</sup>	930 <sup>b</sup>	160 <sup>eg</sup>	125 <sup>cd</sup>	12.97 <sup>fg</sup>
48TA15	381.5 <sup>ab</sup>	36.1 <sup>g</sup>	1050 <sup>a</sup>	82 <sup>g</sup>	115 <sup>de</sup>	13.72 <sup>de</sup>
48TB5	222.0 <sup>ef</sup>	44.7 <sup>cd</sup>	675 <sup>d</sup>	403 <sup>c</sup>	119 <sup>de</sup>	13.43 <sup>ef</sup>
48TB10	283.5 <sup>be</sup>	41.1 <sup>e</sup>	795 <sup>c</sup>	236 <sup>de</sup>	90 <sup>f</sup>	14.30 <sup>c</sup>
48TB15	341.0 <sup>ac</sup>	36.3 <sup>g</sup>	1065 <sup>a</sup>	224 <sup>e</sup>	65 <sup>g</sup>	16.61 <sup>a</sup>

Samples with different superscript letters within each column are significantly different at  $p < 0.05$  according to Duncan's multiple range test; TPrMax, time to reach the maximum pressure of 2,200 mbar; HYDHA, actual hydration of dough used in the test (15% moisture basis); D450, pressure drop from PrMax to pressure after 450 s.

in accordance with the conclusions of [14], showing that the technological parameters used during dough preparation influence the leavening performance more than the semolina characteristics. Generally, the addition of OWP had a negative effect on the leavening volume compared with the respective controls in both 4T and 48T. This result is in accordance with most studies, which have demonstrated that almost every milk fraction, including whey proteins (powders or concentrates) and casein, depresses loaf volume [21, 24]. In our study, the depressing effect was greater in 4T than 48T at the highest OWP concentrations (i.e., A at 15% and B at 10% and 15%; Table 3). Conversely, at the lowest concentration of A (5%), dough made from 4T and 48T showed the same leavening volume as their respective controls. As far as the differences in OWP A and OWP B are concerned, data in Table 3 indicate that the leavening volume of doughs was lower when OWP B was used.

Our data show that the addition of OWP leads to increased dough elasticity, as determined by the contribution of the stress relaxation constant  $C_i$  to PC1 (Figure 2). We observed that the greater the reduction of the leavening volume was, the higher the increase in  $C_i$  values was; for example, in the dough made from 4T and 48T with B at a 15% substitution rate, the  $C_1$  value increased by 151% and 81%, respectively, with respect to their controls. At the same time, the leavening volume decreased by 56% and 52% in the same samples (data not shown). These data suggest that the addition of OWP resulted in a gluten matrix that had a greater resistance to extension, which negatively affected the volume of leavening. Fois et al. [19] reported that samples with a gluten network that is too strong and with a  $P$  to  $L$  ratio that is too high tend to perform worse than samples with normal gluten strength.

In this study, the two kinds of semolina were well separated with respect to the second axes (Figure 2), regardless of the addition of whey protein. Relaxation time  $\tau_i$ , which dominated PC2, clearly distinguished the 4T samples from the 48T samples, indicating that the 48T samples had a greater relaxation time than the 4T samples. The relaxation times can be considered an indication of the relative rates of molecular motion in dissipating stress [25], so the higher the relaxation time, the lower the chain mobility. Lower relaxation times may explain the higher leavening volume in the 4T samples compared with the 48T samples at the lower concentrations of whey proteins (i.e., A at 5% and 10% and B at 5%). Edwards et al. [25] discussed the role of the relaxation time in the baking performance of common and durum wheat. The authors found that dough with lower relaxation times had higher loaf volumes and hypothesized that the strength of the dough is correlated with the relative molecular mobility. In the abovementioned substitution rates, the volume of leavening was ~20% greater in the 4T samples than the homologous doughs made from the strong commercial 48T, and this is consistent with their  $\tau_i$  values.

**3.3. Effect of Whey Powders on the Secondary Structure of Gluten.** FT-IR spectra of dough samples were collected and analyzed to investigate the molecular differences among samples. Results are reported in Table 4. FT-IR spectra of 4TC and 48TC dough in the range of 1780–1440  $\text{cm}^{-1}$  were similar to those found in other studies [17, 26]. Amide I and amide II are the most important bands in the infrared spectrum of proteins. The most intense absorption band is amide I, which was centred at about 1660  $\text{cm}^{-1}$  in this study, and is mainly derived from the C=O stretching of the peptide group combined with N-H bending. The amide

TABLE 4: The effect of OWP on the secondary structure of gluten as revealed by FT-IR. Data are reported as percentages of total area of amide I protein.

Samples	$\alpha$ -Helix	Random coil	$\beta$ -Sheet	$\beta$ -Turn
4TC	12.8 <sup>b</sup>	13.6 <sup>g</sup>	51.7 <sup>bc</sup>	21.9 <sup>b</sup>
4TA5	11.4 <sup>b</sup>	15.6 <sup>de</sup>	53.7 <sup>b</sup>	19.3 <sup>bc</sup>
4TA10	11.3 <sup>b</sup>	14.0 <sup>fg</sup>	57.6 <sup>a</sup>	18.0 <sup>bd</sup>
4TA15	11.7 <sup>b</sup>	15.5 <sup>de</sup>	51.5 <sup>bd</sup>	21.8 <sup>b</sup>
4TB5	12.7 <sup>b</sup>	14.1 <sup>fg</sup>	53.7 <sup>b</sup>	19.4 <sup>bc</sup>
4TB10	12.1 <sup>b</sup>	12.9 <sup>g</sup>	53.7 <sup>b</sup>	21.1 <sup>b</sup>
4TB15	13.1 <sup>b</sup>	15.1 <sup>ef</sup>	51.8 <sup>bc</sup>	20.1 <sup>bc</sup>
48TC	13.6 <sup>b</sup>	11.5 <sup>h</sup>	48.9 <sup>ce</sup>	25.9 <sup>a</sup>
48TA5	16.0 <sup>a</sup>	16.7 <sup>d</sup>	47.8 <sup>ef</sup>	19.4 <sup>bc</sup>
48TA10	16.5 <sup>a</sup>	18.5 <sup>c</sup>	46.7 <sup>ef</sup>	18.2 <sup>bd</sup>
48TA15	17.0 <sup>a</sup>	19.9 <sup>bc</sup>	48.0 <sup>df</sup>	15.1 <sup>d</sup>
48TB5	16.9 <sup>a</sup>	19.7 <sup>bc</sup>	48.3 <sup>cf</sup>	19.8 <sup>bc</sup>
48TB10	17.3 <sup>a</sup>	20.4 <sup>ab</sup>	45.2 <sup>f</sup>	17.2 <sup>cd</sup>
48TB15	17.8 <sup>a</sup>	21.3 <sup>a</sup>	44.7 <sup>f</sup>	17.1 <sup>cd</sup>

Samples with different superscript letters within each column are significantly different at  $p < 0.05$  according to Duncan's multiple range test.

II band was found in the range of 1580–1510  $\text{cm}^{-1}$ ; this band is more complex to study because it is derived from a combination of N-H bending, C-N, and C-C stretching. This is the reason why only the amide I band was studied in depth. The peak assignments to protein conformations in the amide I region were the same as reported by Wang et al. [18]: the bands located at 1650–1660  $\text{cm}^{-1}$  were assigned to the  $\alpha$ -helix, the bands located at 1618–1640  $\text{cm}^{-1}$  and 1670–1690  $\text{cm}^{-1}$  were assigned to the  $\beta$ -sheet, the bands at 1660–1670  $\text{cm}^{-1}$  and 1690–1700  $\text{cm}^{-1}$  were assigned to  $\beta$ -turns, and the band at 1645  $\text{cm}^{-1}$  was assigned to random coils. The results of the curve fitting procedure (Table 4) show that the predominant secondary structure in the dough was the  $\beta$ -sheet conformation (about 50%), as found by other authors [18], followed by  $\beta$ -turn and  $\alpha$ -helix and random coil conformation. We noted a clear separation between the two samples, 4T and 48T, as far as the secondary structure of protein is concerned. In fact, in the 4T samples the percentage of  $\beta$ -sheet structures was generally higher than the 48T samples, and the percentage of  $\alpha$ -helix and random coil structures was lower. The  $\beta$ -sheet structures are indicative of protein aggregation [17, 27], and the increase of  $\beta$ -sheet and  $\beta$ -turn structures is an indicator of dough strength [18]. These data can offer a key to understand the differences in the volume of leavening between the two samples. In fact, as mentioned before, generally the 4T samples showed a higher value of leavening volume than 48T, consistent with their higher content of  $\beta$ -sheet, and lower content of  $\alpha$ -helix and random coil structures. Secondary structures were highly sensitive to the addition of ovine whey powder in the 48T semolina, where there was a significant increase in the  $\alpha$ -helix and random coil structures and a concomitant decrease of the  $\beta$ -turn structures, after the addition of whey powder. We suggest that a technological parameter may have influenced the relative percentage of the secondary structures. The 48T

samples differed from the 4T samples with regard to mixing time (Table 3). It is worth noting that the mixing time was significantly higher in the 48T samples compared with their homologous 4T samples at each percentage of whey powder (Table 3); furthermore the mixing time increased as the amount of OWP increased. The longer mixing time may have been responsible for the disruption of the protein aggregates in the gluten network, as reported by other authors [28, 29], thereby explaining the decrease of the leavening volume in the 48T samples upon OWP addition. This was reflected by the increase in random coil and unordered structures, which increased from 12% in the control 48TC sample to 20% in the 48TA15 (+66%) sample. Notably, the content of  $\beta$ -turn structures, which are highly dependent on the mechanical history of the sample [30, 31], decreased from 26% in the 48TC sample to 19% in the 48TA5 sample, whereas it remained unchanged in the 4T samples.

#### 4. Conclusions

In conclusion, the addition of OWP to semolina had a negative effect on the leavening volume of the dough, except when the OWP A was added at the lowest percentage (5%), which means the lowest addition of whey proteins. In this study, the reduction of leavening volume in dough obtained with two types of semolina, supplemented with OWP, probably occurred because it led to a strong increase in dough elasticity and tenacity, in all samples. In the 48T samples a longer mixing time was needed to reach the sought after consistency on the consistograph, than the 4T, and this was reflected in the higher content of unordered protein structures and lower content of  $\beta$ -sheets in the 48T samples than 4T, as revealed by FT-IR spectra of the doughs. This suggested that the prolonged mixing caused partial disruption of the continuous gluten network.

## Disclosure

This article does not contain any studies with human or animal subjects.

## Conflicts of Interest

The authors have no conflicts of interest to declare.

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