Application of ultrasound for quality control of Torta del Casar cheese ripening

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ABSTRACT

This work aimed to establish the ultrasound parameters **[AU1: A parameter (mean or variance) defines or describes a population in statistical terms. The term "parameter" is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment. Please check usage and revise if needed.]** that can be useful to classify the defects in the soft cheese Torta del Casar during ripening. During ripening by ultrasound, 1 standard and 3 defective cheese batches (anomalous microbial population, inadequate pressing curd, and excessive pressing curd) were evaluated. Ultrasound parameters related to velocity, attenuation, and frequency were calculated and correlated with the physicochemical and rheological properties of the cheeses. Ultrasound data were considered variables in linear discriminant analysis to attempt cheese classification at different periods of the ripening process. Defective soft cheeses could be discriminated from standard ones with good accuracy, mainly at the final stages of ripening. The differentiation of cheese samples from 2 of the defective cheese batches (anomalous microbial population and inadequate pressing curd) from the standard was mainly attributed to different values of the attenuation-related parameters, whereas for samples from the other defective batch (excessive pressing curd), some parameters related to velocity and frequency were responsible for such discrimination.

Key words: Torta del Casar, ultrasound, defect classification, linear discriminant analysis

INTRODUCTION

Torta del Casar is a high-quality Spanish cheese marketed under the Registry of the Protected Designation of Origin Torta del Casar, in accordance with Regulation (EC) 1491/2003 (European Commission, 2003). This type of soft-bodied cheese is made from raw ewe milk using only vegetable coagulant as rennet and without any added starter culture. The absence of a standardizing thermal process means the final characteristics depend on the indigenous microbiota present (Ordiales et al., 2013a,b). In addition, the milk-clotting activity of the traditional vegetable coagulant, prepared using an aqueous infusion of the dried flowers from *Cynara cardunculus* L., presents great variability. Ordiales et al. (2012) showed that the diverse natural populations harvested at different flower ripening stages, mixed with other parts of the plant and dried in different conditions, strongly influence its coagulant properties. This major heterogeneity in Torta del Casar manufacture can negatively affect the quality parameters, including low product yield, and inhomogeneity of relevant sensorial factors between cheese batches, such as texture parameters (Tunick, 2000). Ordiales et al. (2013b) revealed that the uncontrolled growth of heterofermentative lactic acid bacteria (**LAB**) adversely influences the flavor of Torta del Casar. Likewise, the clotting and proteolytic activities of vegetable rennets used in the manufacturing of soft-type cheeses affect both the physicochemical parameters in the initial stages and the texture and sensorial properties of the final product (Ordiales et al., 2014). Thus, technological quality control during the cheese-making process by fast, reliable methods would be desirable for standardization of this high-quality product.

Highlighted among the nondestructive techniques for assessing food properties are small-displacement probes; vibration rheometers; visible-, near-, and mid-infrared

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spectroscopy; z-nose (advanced electronic nose); nuclear magnetic resonance spectroscopy; electronic noses; computer vision; and ultrasound (Benedito et al., 2006). Ultrasound-based technologies have been proposed as a quality control tool not only to determine the optimum cut time during cheese manufacture (Koc and Ozer, 2008; Jiménez et al., 2017) but also to detect process anomalies in-line (Telis-Romero et al., 2011). Other researchers used contact or noncontact ultrasound to detect defects and internal objects in cheeses (Lee et al., 1992; Hæggström and Luukkala, 2001; Leemans and Destain, 2009) and to evaluate the texture of cheeses such as Cheddar, Mahon, and Manchego (Benedito et al., 2000, 2002, 2006; Cho et al., 2001; Cho and Irudayaraj, 2003; Nassar et al., 2010). Most studies carried out to detect texture defects in cheese using ultrasound have measured ultrasonic velocity and attenuation at one or more temperatures. In the present work, defective Torta del Casar batches were analyzed by ultrasound during ripening, and several ultrasound parameters related to the velocity, frequency, and attenuation were correlated with the physicochemical and texture parameters of the cheese and used to discriminate these defective batches from standard samples at the ripening temperature.

The objective of this work was to assess the feasibility of ultrasound as a tool for quality control in ripening of Torta del Casar, relating the ultrasonic parameters to the physicochemical and texture characteristics of the cheeses. In addition, ultrasonic parameters were used to discriminate between standard cheeses and defective products throughout the cheese-making process.

MATERIALS AND METHODS

Torta del Casar Cheese-Making Procedure

The cheeses were made with a mixture of raw milk obtained from the Merino and Entrefino breeds. The milk was heated in a 100-L stainless-steel vat to 28 to 30°C and clotted by adding 10 mL of the aqueous extract from flowers of the cardoon *C. cardunculus* L. per liter of milk, prepared by macerating 50 g of dried flowers in 1 L of water for 24 h. After the curd formed, it was broken at 30°C into rice grain–sized pieces using fine metallic threads agitated for 10 min, followed by 10 min of rest and 10 min of stirring. Afterward, it was transferred to suitable molds and pressed for 2 h at an initial pressure of 2 kg/cm^2, which was increased by 0.5 kg/cm^2 every 30 min. Once pressed, the cheese was submerged in brine (16% NaCl, wt/vol) for 4 h. Ripening was controlled at 5 to 7°C and relative humidity >90% for 70 d. Four batches of Torta del Casar cheese were prepared (Supplemental Figure S1, https://doi.org/10 .3168/jds.2020-18160), introducing a specific modification during the cheese-making procedure, as follows:

- Standard batch (**ST[AU2: JDS avoids single-letter abbreviations. Therefore, S has been changed to ST throughout.]**): this cheese was manufactured by following the general cheese-making procedure.
- Pressing defect batch 1 (**PD1**): for the preparation of body mechanical hole defects, cold water was used instead of hot water in the double-jacketed vat during the stirring step. With this cheese-making modification, grains become cold when the curd is shaped, causing ineffective binding among the various curd parts.
- Pressing defect batch 2 (**PD2**): the curd was broken into rice grain–sized pieces, as indicated in the general cheese-making procedure, but the vat was set at 35°C rather than 30°C to achieve a higher moisture in the paste.
- Microbial defect batch (**MD**): the cheese with early blowing defects was prepared by adding 3.61 g of lyophilized inoculum to 96 L of milk tempered at 30°C before clotting. The inoculum comprised proteolytic and potential gas-producing microorganisms, including *Escherichia coli* (2.07×10^{10} cfu/g), *Enterobacter aerogenes* (3.2×10^{10} cfu/g), and *Kluyveromyces lactis* (1.19×10^{10} cfu/g).

A total of 30 to 32 units were obtained for each batch with approximately 950 g as initial weight (diameter: 13 cm; height: 7 cm). Three cheeses were randomly chosen from each batch for analysis after 0, 2, 4, 6, 8, and 10 wk of ripening.

Physicochemical Parameters

Dry matter (%), total protein, and total fat were analyzed using standard methods 5534:2004 (ISO, 2004), 3433:2008 (ISO, 2008), and 8968-1:2014 (ISO, 2014), respectively. Ash content was determined by incineration in a muffle furnace at 550°C (AOAC International, 2000). The pH was determined using a Crison 2002 pH meter (Crison Instruments, Barcelona, Spain). In addition, each cheese was weighed, and the diameter and height were measured using a caliper. Density was calculated, assuming a cylindrical geometry.

Microbial Count

Ten-gram aliquots of each cheese sample were aseptically transferred to sterile plastic pouches for microbial enumeration. After 10-fold dilution with 1% peptone water (Pronadisa, Alcobendas, Madrid, Spain), each

sample was homogenized for 120 s using a Stomacher Lab Blender 400 (Seward Ltd., London, UK). Serial 10-fold dilutions were prepared from the same solution and inoculated onto agar plates. The LAB were grown on de Man, Rogosa and Sharpe agar (Oxoid, Hampshire, UK) acidified to pH 5.6 with acetic acid (10%) under anaerobic conditions at 30°C for 48 h. Mold and yeast counts were determined on oxytetracycline–glucose–yeast extract agar (Oxoid) at 25°C for 5 d, counting typical colonies. Violet red bile–glucose agar (Oxoid) was used for *Enterobacteriaceae* counts at 30°C for 24 h.

Texture

A TA.XTplus texture analyzer, fitted with a 5-kg load cell (Stable Micro Systems, Godalming, UK) and spherical stainless-steel probe (P/1S), was used to perform a texture compression analysis of the cheeses throughout the ripening process. Cheeses were removed from storage at 5°C, positioned under the probe, and measured on both sides (top and bottom). The operational parameters were as follows: pretest speed, 3 mm/s; test speed, 2.00 mm/s; posttest speed, 10.00 mm/s; distance, 10.0 mm; and trigger force, 25 g. From the resulting graphs, the values for firmness and stickiness of cheese were analyzed using the installed Exponent software version 3.0.5.0**[AU3: Please add URL or name and location of manufacturer.]**.

In addition, a texture spreadability analysis was undertaken after approximately 4 wk of ripening, when the Torta del Casar had acquired a creamy appearance, using a HDP/SR**[AU4: Please spell out HDP-SR.]** spreadability probe. The samples were packed into the lower cone with a spatula and measured at 20°C. The operational parameters were as follows: pretest speed, 3 mm/s; test speed, 2.00 mm/s; posttest speed, 10.00 mm/s; distance, 10.0 mm; and trigger force, 25 g. From the resulting graphs, the maximum force (the point at which the probe was at its maximum penetration depth) was calculated using the installed Exponent software version 3.0.5.0 and described as unctuosity.

Ultrasonic Parameters

The cheeses were inspected ultrasonically using contact techniques in through-transmission mode. The measurement setup is illustrated schematically in Figure 1A. To reduce the operator risk, the transducers were mounted on a custom-designed metal structure that ensured their perfect face-to-face alignment (Figure 2). The range of frequencies used in the study of cheese specimens was extended by using 2 different pairs of shear wave transducers of 250- and 500-kHz frequencies, respectively (Olympus Panametrics NDT,

Waltham, MA). Table 1 lists the principal characteristics of the different transducers used, including the near-field zone and the beam spread angle. Both of these values depend on the propagation medium, so Table 1 also provides approximate values for cheeses, considering a mean ultrasonic pulse velocity (**UPV**) of 750 m/s for shear waves. The pulser-receiver models 5058PR and 5077PR (Olympus Panametrics NDT) were used for emission and reception of the ultrasonic signals with transducers V150 and V151, respectively. For the acquisition and digitization of the signals, the pulser-receiver units were connected to an oscilloscope (100-MHz bandwidth, 1-GSa/s sample rate, 2.5k points record length; Tektronix TM TDS1012B, Wilsonville, OR). The Tektronix OpenChoice TDSPCS1 software package was used for subsequent data processing and analysis.

The shear UPV (UPV_S) values obtained with the respective transducers were calculated based on the time lapse from the signal's emission until its recep-
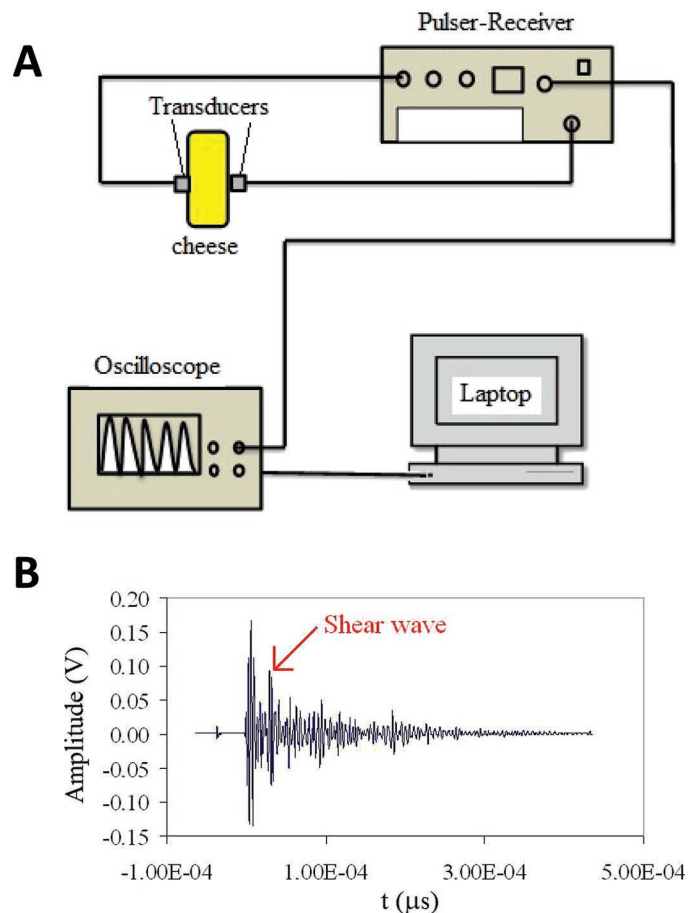

Figure 1. (A) Scheme of the setup used for the ultrasound inspection of the cheeses, and (B) typical A-scan obtained with one of the shear wave transducers**[AU20: Define t on x-axis in panel B.]**.
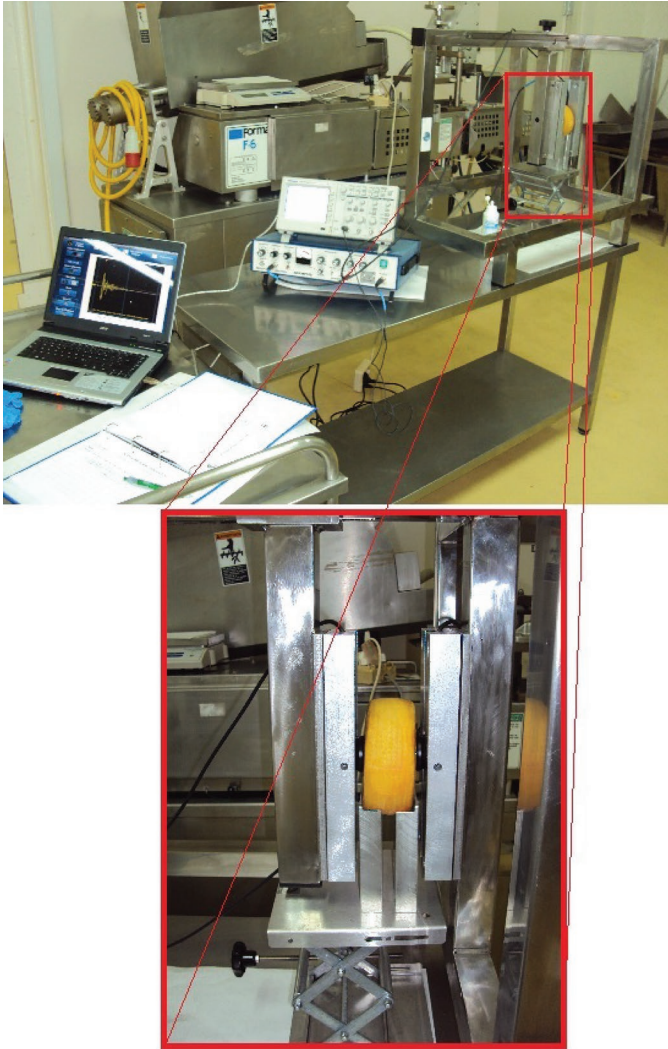
Figure 2. Custom-designed metal structure to ensure the perfect face-to-face alignment of the cheese.

shows a typical A-scan obtained with one of the shear wave transducers. From UPV_L, UPV_S, and the apparent densities or specific weights (ρ) of each sample, the following constants were calculated for each piece of cheese according to the expressions established by the American Society for Testing and Materials (2008) in protocol ASTM D2845-08.

Young's modulus (**E**):

$$E = \frac{\rho \times UPV_S^2 \times \left(3UPV_L^2 - 4UPV_S^2\right)}{UPV_L^2 - UPV_S^2}. \quad [1]$$

Shear modulus (μ):

$$\mu = \rho \times UVP_S^2. \quad [2]$$

Bulk modulus (**k**):

$$k = \frac{\rho \times \left(3UPV_L^2 - 4UPV_S^2\right)}{3}. \quad [3]$$

Lamé's constant (λ):

$$\lambda = \rho \times \left(UPV_L^2 - 2UPV_S^2\right). \quad [4]$$

Poisson's ratio (ν):

$$\nu = \frac{\left(UPV_L^2 - 2UPV_S^2\right)}{2 \times \left(UPV_L^2 - UPV_S^2\right)}. \quad [5]$$

tion, called the time of flight (**TOF**). From the known distance (d) velocity was determined: UPV = d/TOF. In addition, with the shear wave transducer values, the received signal has an initial longitudinal wave train, generated by mode conversion. These particular longitudinal waves (**UPV_L**) were also obtained. Figure 1B

Fast Fourier transform (**FFT**) frequencies corresponding to the 25th, 50th, 75th, and 99th percentiles of the received signals in the cumulative frequency periodograms of the FFT, as well as the attenuation quantified in terms of the time taken to receive the energy (10, 20, 30, 40, 50, 60, 70, 80, and 90% of the total energy received), expressed as a multiple of the TOF, were also recovered (Cerrillo et al., 2014). These parameters were

Table 1. Characteristics of the different transducer models used[1]

Model[2]	Diameter (m)	Central frequency (kHz)	−6 dB bandwidth (%)	Near-field length (cm)	Beam angle (°)
V150	0.025	250	92	5.21	8.41
V151	0.025	500	101	10.42	4.20

[1]The value ultrasonic pulse velocity = 750 m/s was applied in the calculation of near-field length and beam angle.

[2]The pulser-receiver models 5058PR and 5077PR (Olympus Panametrics NDT, Waltham, MA) were used for emission and reception of the ultrasonic signals with transducers V150 and V151, respectively.

determined along the center of opposite faces of the specimens for each of the selected inspection frequencies.

Statistical Analysis

Data were statistically analyzed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). Descriptive statistics of the ultrasonic parameters were determined, and the evolution of the physicochemical, microbial, and texture parameters of the cheese batches was studied using 1-way ANOVA and separated by Tukey's honest significant difference test ($P \leq 0.05$). The efficiency of the selected ultrasonic parameters as control markers of physicochemical and texture parameters of the cheese specimens was evaluated by Pearson correlation coefficients. For classification of cheese batches during their ripening, linear discriminant analysis (**LDA**) was applied to the ultrasonic data. The discriminant variables for each ripening interval evaluated (2–4, 4–6, 6–8, and 8–10 wk) were identified by a step-wise algorithm based on the Wilks' lambda method. The results of the LDA classification model with best performance were graphically represented by projecting the cheese groups into the space of discriminant functions (**DF**).

RESULTS AND DISCUSSION

Physicochemical and Texture Parameters

Table 2 presents the physicochemical and texture parameters of the cheese batches during the ripening process. In general, DM, total protein, and total fat increased during the ripening process, with ranges of 45.1 to 59.6, 11.7 to 19.3, and 23.0 to 33.6%, respectively, without differences among the batches studied at the end of ripening. In the same way, the results for ash, pH, and density did not show any relevant differences among the studied batches during the ripening process. These physicochemical results were similar to those obtained previously for Torta del Casar and other similar soft-bodied cheeses (Roa et al., 1999; Sanjuán et al., 2002; Ordiales et al., 2013a). In contrast, texture data were more variable between batches. The firmness of the standard batch (ST) increased until the end of ripening, whereas for batch PD1, this tendency was not evident. In batch MD, maximal firmness occurred at 6 wk after ripening and then declined continuously. For batch PD2, this parameter was particularly high throughout ripening. Delgado et al. (2010) found a reduction of cheese hardness during the ripening of Torta del Casar, which is characteristic of this type of cheese. In this study, cheeses were previously opened by cutting the whole upper rind to evaluate the texture of the cheese paste. Data on the other measured texture parameters revealed the highest values for unctuosity and stickiness for batch MD. The effect of microbial population on the rheology properties of this type of cheese has been described by Pereira et al. (2010). The relationship between textural and proteolytic parameters has been attributed to the degradation of the casein matrix, decreasing the hardness and consistency of Torta del Casar and increasing its adhesiveness during ripening (Delgado et al., 2010). Conversely, batch PD1 showed lowest values for unctuosity and stickiness.

Evolution of Microbial Population During Cheese Ripening

The mean counts of LAB during ripening were greater than 8 log cfu/g for all batches without significant differences (Figure 3). Yeast counts were essentially constant until wk 6 of ripening, with most values ranging from 4 log cfu/g (PD2) to 6 log cfu/g (MD and PD1). At the end of ripening, 3 batches showed a noticeably decreased yeast count, with values close to 4 log cfu/g for MD and 3 log cfu/g for ST and PD2. A similar evolution was observed for the *Enterobacteriaceae* population, with counts stable around 8 cfu/g in all batches during the initial 4 wk of ripening and a progressive decrease in the second period. However, batches ST and PD2 exhibited a more pronounced decline in this microbial group relative to batch PD1. The counts of LAB, yeast, and *Enterobacteriaceae* found in this study are similar to those previously reported in this type of cheese (Pintado et al., 2010; Ordiales et al., 2013a). Lactic acid bacteria appear to be the predominant microbial group in Torta del Casar (Mas Mayoral et al., 1991), in which they eventually reach viable numbers of 8 log cfu/g by the time of consumption (Ordiales et al., 2013a). In addition, the presence of high numbers of *Enterobacteriaceae* is not unusual in soft cheese made from raw milk (Alegría et al., 2009; Gonçalves et al., 2018). These high levels of microorganisms during ripening suggest a relevant contribution of their associated activity in the development of the sensory characteristics of Torta del Casar cheese, including its texture.

Ultrasonic Parameters

The evaluation of the properties of food, including different types of cheese, mainly occurs through the analysis of the ultrasonic velocity as the simplest and the most reliable measurement (Benedito et al., 1998; Gallo et al., 2018). Benedito et al. (2000) noticed an increase in ultrasonic velocity during ripening of the firm-textured Cheddar cheese, ranging from 1,657 to 1,677 m/s at 12°C and from 1,684 to 1,693 m/s at 5°C

Table 2. Physicochemical and texture parameters of Torta del Casar cheese batches during ripening

Batch and week	Physicochemical parameter						Texture parameter		
	DM (%)	Total protein (%)	Fat (%)	Ash (%)	pH	Density (g/cm³)	Firmness (g)	Unctuosity (g)	Stickiness (g)
Standard (ST)									
0	$45.1^{D,b}$	12.5^C	23.0^D	2.22	6.69^A	$1,074^{A,a}$	$853^{C,b}$	—	—
2	$46.2^{D,c}$	$16.1^{B,a}$	$24.1^{D,c}$	2.21	$5.17^{B,b}$	$1,051^{AB,a}$	$878^{C,b}$	—	—
4	$48.7^{C,b}$	$16.2^{B,ab}$	$27.7^{C,c}$	2.27	$4.73^{B,b}$	$1,000^B$	$787^{C,c}$	1,918	−1,736
6	$53.5^{B,c}$	$17.2^{AB,a}$	$30.3^{B,b}$	2.37	5.03^B	$1,047^{AB,a}$	$1,387^{B,c}$	$1,652^c$	$-1,543^a$
8	$54.3^{B,c}$	$18.3^{AB,a}$	$30.9^{B,b}$	2.33	5.18^B	$939^{C,b}$	$1,608^{B,b}$	$1,597$	$-1,731^a$
10	58.1^A	19.3^A	$33.3^{A,ab}$	2.51	5.24^B	$951^{C,b}$	$2,252^{A,a}$	$1,626^b$	$-1,632^a$
Defective pressure (PD1)									
0	$46.2^{D,b}$	11.7^D	24.6^D	2.34	6.73^A	$1,011^b$	$1,090^{A,b}$	—	—
2	$53.6^{C,a}$	$13.7^{C,b}$	$30.9^{C,a}$	2.44	$5.50^{C,a}$	997^b	$774^{B,b}$	—	—
4	$54.8^{BC,ab}$	$15.7^{BC,ab}$	$32.2^{BC,a}$	2.18	$5.50^{B,a}$	970	$923^{AB,c}$	—	—
6	$55.1^{BC,bc}$	$14.6^{C,b}$	$34.6^{A,a}$	2.61	5.48^B	990^b	$857^{AB,d}$	$1,895^{B,ab}$	$-2,060^{A,a}$
8	$55.3^{B,c}$	$18.4^{A,a}$	$33.0^{AB,a}$	2.42	5.40^B	999^a	$862^{AB,c}$	$2,336^B$	$-2,631^{A,b}$
10	58.4^A	17.5^{AB}	$33.0^{AB,ab}$	2.72	5.47^B	$1,001^a$	$1,073^{A,c}$	$3,477^{A,a}$	$-3,755^{B,c}$
Excessive pressure (PD2)									
0	$48.3^{E,a}$	10.8^C	23.3^C	2.49	6.68^A	$1,013^b$	$4,242^{A,a}$	—	—
2	$52.9^{D,ab}$	$13.2^{BC,b}$	$24.2^{C,c}$	3.02	$5.37^{B,b}$	$1,028^{ab}$	$4,040^{A,a}$	—	—
4	$57.1^{C,a}$	$14.9^{B,b}$	$29.0^{B,bc}$	3.10	$5.04^{B,ab}$	$1,006$	$3,658^{B,a}$	—	—
6	$59.6^{B,a}$	$15.7^{AB,ab}$	$31.2^{A,b}$	3.31	5.04^B	$1,008^{ab}$	$3,600^{B,a}$	$8,169^{A,a}$	$-5,586^{B,b}$
8	$63.2^{A,a}$	$10.7^{C,b}$	$31.7^{A,ab}$	3.30	4.97^B	987^{ab}	$4,291^{A,a}$	$2,281^B$	$-2,117^{A,ab}$
10	59.0^B	17.8^A	$31.7^{A,b}$	3.17	5.24^B	$1,011^{ab}$	$3,728^{B,c}$	$3,173^{AB,a}$	$-2,521^{A,b}$
Anomalous microbial population (MD)									
0	$48.0^{D,a}$	11.7^C	24.0^D	2.06	6.55^A	$1,022^b$	$823^{C,b}$	—	—
2	$51.8^{C,b}$	$13.0^{B,b}$	$28.0^{C,b}$	2.21	$5.99^{B,a}$	$1,019^{ab}$	$791^{C,b}$	—	—
4	$54.9^{B,ab}$	$17.9^{A,a}$	$30.3^{B,b}$	2.39	$5.55^{B,a}$	972	$1,551^{B,b}$	—	—
6	$55.4^{B,b}$	$16.2^{A,ab}$	$29.5^{BC,b}$	2.37	5.54^B	979^b	$2,277^{A,b}$	$2,818$	$-1,755^a$
8	$57.7^{A,b}$	$17.0^{A,a}$	$32.5^{A,ab}$	2.40	4.80^C	985^{ab}	$1,612^{B,b}$	$1,892^{bc}$	$-2,085^{ab}$
10	57.6^A	18.0^A	$33.6^{A,a}$	2.31	5.49^C	982^{ab}	$1,690^{B,b}$	$1,812^b$	$-1,917^{ab}$

A–EMean values within a column of the same batch with different uppercase superscripts are significantly different during processing ($P < 0.05$).

a–dMean values within a column of the same processing week with different lowercase superscripts are significantly different between batches ($P < 0.05$).
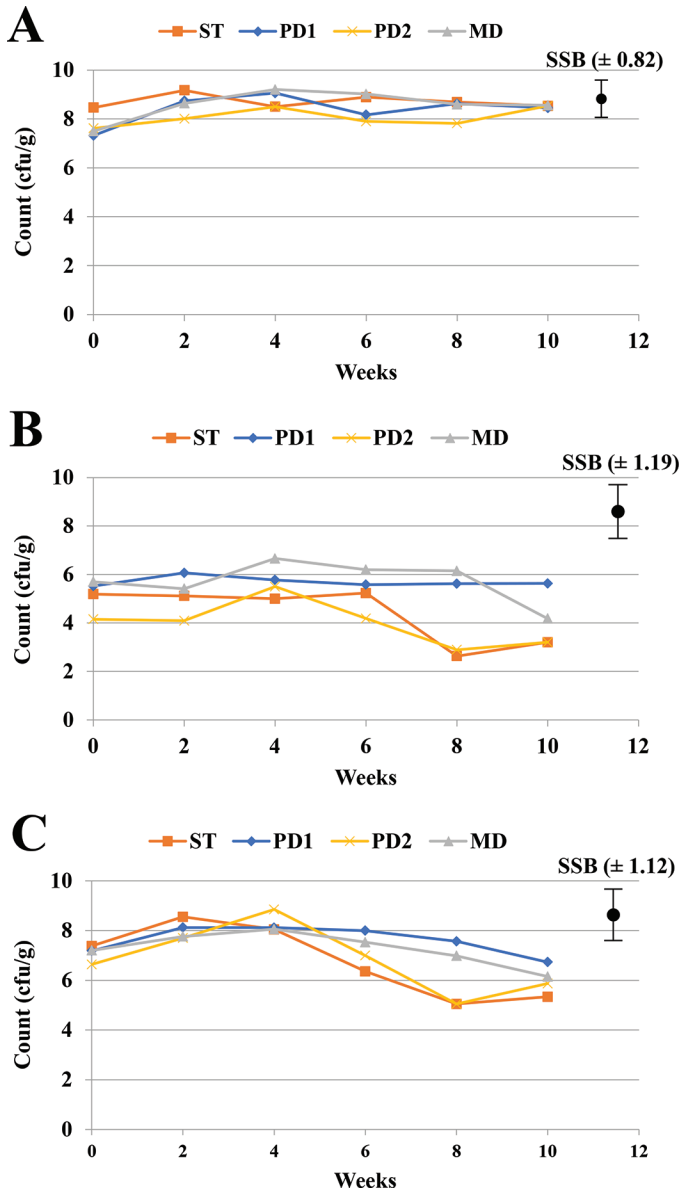
Figure 3. Evolution of (A) lactic acid bacteria, (B) yeast, and (C) *Enterobacteriaceae* counts during the ripening of the cheese batches studied: standard (ST), defective pressure (PD1), excessive pressure (PD2), and anomalous microbial population (MD). SSB = statistical significance bar using Tukey's honestly significant difference test.

(transducers of 1 MHz, 1.9-cm crystal diameter; model A314S-SU, Panametrics NDT). These values are more constant than those found in this study for Torta del Casar, a soft-textured cheese, with ranges of 1,144 to 1,723 and 1,246 to 1,547 m/s for transducers V150 and V151, respectively (Table 3).

Several parameters related to velocity, frequency, and attenuation were studied in this work. In addition to ultrasound velocity in the longitudinal (**VL**) and transverse (**VT**) directions and the velocity ratio (VT/

VL), the elastic constants were calculated from the ultrasonic velocity measurements. From these parameters, VL showed the lowest relative standard deviation (3.71–5.18%), whereas Lamé's constant and v presented the highest coefficients of variation (40.37–48.47 and 30.37–38.08%, respectively). In all cases, the level of dispersion of the parameters related to the velocity was higher with transducer V150 than with V151. On the contrary, the relative standard deviation values of the highest amplitude frequency and the FFT frequencies corresponding to the 25th, 50th, 75th, and 99th percentiles of the received signals were higher for transducer V151 than for V150, mainly for the highest amplitude frequency, showing more than 40% of the variability (Table 3). Regarding the attenuation-related parameters, the dispersion data were slightly higher with transducer V150 than with V151, with AT 60**[AU5: Please define the AT terms used in the text (AT 60, AT 70, AT 90, and so on).]** showing the highest coefficients of variation for both transducers (35.6 and 31.2%, respectively). The high variability found for some ultrasound parameters means that those could show a good response to the changes and variations in the physicochemical and texture parameters associated with the cheese defects or ripening process.

Relationship Between Physicochemical and Texture Properties with Ultrasonic Parameters During Ripening of Soft Cheese Torta del Casar

Correlations of the ultrasound parameters obtained using both transducers V150 and V151 with the physicochemical and texture properties of the Torta del Casar cheese batches during ripening are shown in Table 4. These results evidenced that transducer V150 performed better than transducer V151. Using transducer V150, the ultrasonic parameters presented a strong correlation ($P < 0.01$) with several physicochemical and texture parameters. The values of most velocity-related parameters and elastic constants were negatively correlated (positive in the case of v) with the fat in DM of the cheese samples during ripening, particularly for VT, E, and Shear modulus (Table 4). This inverse correlation between the fat content of food and ultrasound velocities (VL and VT) is a well-recognized phenomenon. Consequently, these parameters have been used to predict the fat percentage in meat products, fish, and different types of cheese (Park et al., 1994; Simal et al., 2003; Shannon et al., 2004; Telis-Romero et al., 2011). Conversely, the fat content in DM of Torta del Casar was also the most important physicochemical parameter for explaining the attenuation behavior. Concretely, AT 70 showed the highest correlation with fat in DM (Table 4). This indicates

Table 3. Descriptive statistics of ultrasonic parameters measured in Torta del Casar cheese batches during processing using transducers V150 and V151[1]

Ultrasonic parameter	Transducer V150					Transducer V151				
	Mean	SD	RSD[2]	Minimum	Maximum	Mean	SD	RSD	Minimum	Maximum
Velocity[3]										
VL (m/s)	1,496	77	5.18	1,144	1,723	1,434	53	3.71	1,246	1,547
VT (m/s)	833	132	15.83	533	1,202	797	93	11.70	605	1,079
VT/VL ratio	0.56	0.08	14.55	0.36	0.76	0.56	0.07	11.70	0.43	0.74
Young's modulus	1.74E+09	3.93E+08	22.56	8.01E+08	2.53E+09	1.61E+09	2.62E+08	16.22	9.80E+08	2.31E+09
Poisson's ratio	0.26	0.10	38.08	0.006	0.423	0.27	0.082	30.37	0.0167	0.388
Bulk modulus	1.32E+09	3.06E+08	23.22	7.03E+08	2.11E+09	1.20E+09	2.55E+08	21.21	5.11E+08	1.69E+09
Shear modulus	7.12E+08	2.23E+08	31.36	2.83E+08	1.60E+09	6.48E+08	1.51E+08	23.27	3.54E+08	1.10E+09
Lamé's constant	8.48E+08	4.11E+08	48.47	1.22E+07	1.83E+09	7.87E+08	3.18E+08	40.37	3.24E+07	1.40E+09
Frequencies[4] (Hz)										
Highest amplitude frequency	278,404	39,596	14.22	180,201	371,000	284,901	114,252	40.10	138,952	625,860
FFT$_{25}$	246,563	23,415	9.50	180,472	305,940	262,719	56,438	21.48	175,834	461,402
FFT$_{50}$	283,546	22,298	7.86	185,100	352,147	352,947	85,409	24.20	204,267	578,995
FFT$_{75}$	318,158	22,021	6.92	261,453	401,160	470,989	86,125	18.29	290,513	663,735
FFT$_{99}$	517,473	52,070	10.06	347,222	633,528	758,842	59,168	7.80	637,765	1,068,455
Attenuations[5]										
AT 10	1.4	0.3	23.2	1.1	2.8	1.4	0.3	19.8	1.0	2.2
AT 20	1.6	0.5	28.9	1.1	3.2	1.6	0.4	25.9	1.1	3.1
AT 30	1.8	0.6	31.6	1.1	3.3	1.8	0.5	27.9	1.1	3.2
AT 40	2.0	0.7	33.8	1.1	3.8	2.0	0.6	30.8	1.1	3.4
AT 50	2.2	0.8	35.5	1.2	4.2	2.3	0.7	31.1	1.1	4.2
AT 60	2.5	0.9	35.6	1.2	4.5	2.6	0.8	31.2	1.1	4.7
AT 70	2.8	1.0	35.2	1.2	5.1	3.0	0.9	30.6	1.4	6.0
AT 80	3.3	1.1	33.5	1.3	6.2	3.5	1.0	28.6	1.7	6.1
AT 90	4.2	1.3	31.6	1.6	7.6	4.4	1.2	27.3	2.4	7.7

[1]The pulser-receiver models 5058PR and 5077PR (Olympus Panametrics NDT, Waltham, MA) were used for emission and reception of the ultrasonic signals with transducers V150 and V151, respectively.

[2]Relative standard deviation expressed as a percentage.

[3]VL = ultrasound velocity in the longitudinal direction; VT = ultrasound velocity in the transverse direction.

[4]xxx**[AU10: Please define the FFT terms (FFT25, FFT50, and so on).].**

[5]xxx**[AU11: Please define the AT terms (AT 10, AT 20, and so on).].**

that when fat in DM increases, a given percentage of energy is received in a longer time (i.e., the attenuation decreases). Considering that the cheeses are less attenuating when the AT 70 value is greater, this result suggests that the most attenuating Torta del Casar cheese specimens are those with a lower ratio of fat in DM. In heterogeneous products, such as meat and fish, the attenuation coefficient is mainly associated with the scattering contribution (Buckin et al., 2002). In these cases, attenuation is much more sensitive to changes in the structure of the tissue than to changes in composition, which limits their use in composition analysis (Sigfusson et al., 2001; Awad et al., 2012). In semi-hard and hard cheeses, such as Manchego cheese, the number and size of holes of a particular cheese can mask the possible changes in attenuation during maturation (Benedito et al., 2001, 2006). However, attenuation has been proposed as a useful indicating parameter for detecting intramuscular fat (marbling) in homogeneous meat pieces, such as longissimus muscle (Park et al., 1994). In our study, Torta del Casar is a soft-type cheese with a relatively homogeneous paste, so both the dispersion and absorption by casein micelles and fat globules can be considered to provide a noticeable contribution to the attenuation. The attenuation coefficient due to scattering plays the same role as an intrinsic absorption coefficient in cheeses with a weak gas cavity concentration (Nassar et al., 2010).

A strong negative correlation between the density and both total fat and fat in DM of the studied cheeses was confirmed ($P < 0.01$). It can explain, at least partly, the lower values of the ultrasonic attenuation for denser cheese samples, whereas k and Lamé's constant showed a positive correlation with the density of the cheeses (Table 4). However, as mentioned above, good correlations have already been found between penetrated acoustic signals and the density of porous food products (Awad et al., 2012), such as Torta del Casar.

Regarding the ash content, which mainly constitutes chloride and calcium salts, it was correlated with VL and E (Table 4). These results corroborate those recorded by Dukhin et al. (2005), who described the ultrasound parameters related to the speed of sound as the better parameters for investigating effects on a molecular scale, whereas attenuation was considered more suitable for characterizing effects related to the heterogeneity and phase composition of the particular dairy product system, containing casein micelles and fat globules.

Among the texture properties of cheese, firmness was significantly related to the VL, as well as the attenuation coefficient, which showed a high negative correlation at AT 90 (Table 4). Again, this result would indicate a greater firmness of Torta del Casar cheese samples with more ultrasound attenuation and, consequently, with higher propagation speeds. Benedito et al. (2000) described the firmness as the textural parameter best related to the ultrasonic velocity during the ripening of cheeses. In general, cheese maturation increases firmness, thereby increasing the ultrasonic velocity, whereas the attenuation coefficient can show a relatively high variability due to porosities or holes in the cheese samples (Benedito et al., 2002). However, in other food gels with bland taste and porous texture, such as tofu, the ultrasonic attenuation has been used as an alternative to textural or rheological analysis in gel characterization (Ting et al., 2009). In contrast, the results obtained for unctuosity seem to indicate that only ultrasonic parameters related to velocity and frequency would be reliable to determine this textural property of Torta del Casar. Concretely, the unctuosity values showed a strong positive correlation with the values of VT, E, and Shear modulus but negative correlation with v and FFT_{99}**[AU6: Please define FFT terms (FFT99, FFT75, and so on).]**.

These results suggest the efficacy of these ultrasonic parameters as tools for the development of discriminative models during Torta del Casar ripening based on their relationship with some physicochemical parameters (mainly fat in DM and density) and texture properties (firmness and unctuosity) of the cheese during ripening.

Discrimination of Soft Cheese Defects by Ultrasound Parameters During Ripening

The LDA was developed using ultrasonic parameters determined during the ripening of the different batches studied. Leave-more-out cross-validation with 4 cancellation groups was used for testing the predictive capability of the LDA. The results of the LDA for classification of different defects in Torta del Casar cheese as a function of the ripening duration are summarized in Table 5, which displays the ultrasonic variables selected by the stepwise algorithm per ripening phase, ordered according to decreasing importance and the number (and percentage) of correct classifications in both calibration and prediction. The LDA of the ultrasound data collected during the cheese ripening confirms the role of some parameters related to attenuation and velocity as useful tools for the discrimination of the types of cheese studied, besides revealing the discrimination potential of the FFT_{75}. Regarding the attenuation parameters, AT 90 and AT 10 are among the discriminant variables of cheese at 2 to 4 wk of ripening. In contrast, for the 4 to 6- and 6 to 8-wk intervals of ripening, AT 50 appears

Table 4. Physicochemical and texture parameters significantly correlated ($P < 0.01$) with ultrasonic parameters during the processing of Torta del Casar[1]

Ultrasonic parameter	Transducer V150						Transducer V151					
	Physicochemical parameter				Texture		Physicochemical parameter				Texture	
	Fat	Fat in DM	Ash	Density	Firmness	Unctuosity	Fat	Fat in DM	Ash	Density	Firmness	Unctuosity
Velocity[2]												
VL (m/s)		−0.426	0.592		0.500						0.439	
VT (m/s)		−0.582				0.533						
VT/VL ratio		−0.452										
Young's modulus		−0.565	0.389			0.532						−0.535
Poisson's ratio		0.400				−0.511						0.530
Bulk modulus				0.342						0.307		
Shear modulus		−0.532		0.227		0.556						
Lamé's constant												
Frequencies[3] (Hz)												
FFT99						−0.519						
Attenuations[4]												
AT 10		0.379		−0.240	−0.374			0.429				
AT 20		0.488		−0.203	−0.408							
AT 30		0.552		−0.202	−0.441			0.420				
AT 40	0.369	0.592		−0.209	−0.467							
AT 50		0.607		−0.228	−0.505			0.420				
AT 60		0.612		−0.235	−0.512			0.426				
AT 70		0.613		−0.211	−0.519							
AT 80		0.587		−0.201	−0.528							
AT 90		0.579			−0.592				−0.446			

[1]The pulser-receiver models 5058PR and 5077PR (Olympus Panametrics NDT, Waltham, MA) were used for emission and reception of the ultrasonic signals with transducers V150 and V151, respectively.

[2]VL = ultrasound velocity in the longitudinal direction; VT = ultrasound velocity in the transverse direction.

[3]xxx**[AU12: Please define the FFT99.]**.

[4]xxx**[AU13: Please define the AT terms (AT 10, AT 20, and so on).]**.

Table 5. Performance in calibration and prediction of linear discriminant analysis classification applied to the Torta del Casar cheese samples during processing**[AU14: Please provide a more descriptive title so that table can be understood independently of text. Please also revise the "Count" heading to be more specific.]**

Processing period (wk)	Selected variables[1]	Count[2]	Cheese batch[3]				
			ST	PD1	PD2	MD	Total
2–4	AT 90	n	20	18	23	16	77
	VL (m/s)	Computed classes					
	AT 10	Class	14	12	18	10	54
		%	70.0	66.7	78.3	62.5	70.1
		Predicted classes					
		Class	14	10	18	12	54
		%	70.0	66.7	78.3	62.5	70.1[4]
4–6	AT 50	n	16	14	16	11	57
	VT (m/s)	Computed classes					
	FFT$_{75}$ (Hz)	Class	7	11	14	9	41
		%	43.8	78.6	87.5	81.8	71.9
		Predicted classes					
		Class	9	7	14	11	41
		%	56.3	78.6	87.5	63.6	71.9[4]
6–8	AT 50	n	12	9	11	8	40
	FFT$_{75}$ (Hz)	Computed classes					
	Bulk modulus	Class	10	9	11	6	36
	Poisson's ratio	%	83.3	100	100	75.0	90.0
		Predicted classes					
		Class	10	7	11	3	31
		%	83.3	77.8	100	37.5	77.5[4]
8–10	Young's modulus	n	8	5	8	4	25
	VT (m/s)	Computed classes					
	Poisson's ratio	Class	8	5	8	2	23
	AT 40	%	100	100.0	100	50	92.0
		Predicted classes					
		Class	8	5	7	0	20
		%	100	100	87.5	0.0	80.0[4]

[1]VL = ultrasound velocity in the longitudinal direction; VT = ultrasound velocity in the transverse direction**[AU15: Please define the FFT and AT terms.]**.

[2]Class = number of cases correctly classified; % = percentage of cases correctly classified.

[3]ST = standard batch; PD1 = defective pressure batch; PD2 = excessive pressure batch; MD = microbial defect batch.

[4]xxx**[AU16: JDS does not permit bold in tables, so bolding has been converted to a footnote. Please define what bolding indicated.]**.

Table 6. Performance in calibration and prediction of linear discriminant analysis classification applied to the Torta del Casar cheese samples after 6 to 8 and 8 to 10 wk of processing considering the batches PD1 and MD one sample group**[AU17: Please provide a more descriptive title so that table can be understood independently of text. Please also revise the "Count" heading to be more specific.]**

Processing period (wk)	Selected variables[1]	Count[2]	Cheese batch[3]			
			ST	PD2	PD1+MD	Total
6–8	FFT$_{75}$ (Hz)	n	12	12	17	41
	AT 50	Computed classes				
	Poisson's ratio	Class	11	12	15	37
	Bulk modulus	%	91.7	100.0	88.2	92.7
		Predicted classes				
		Class	10	12	15	37
		%	83.3	100.0	88.2	90.2[4]
8–10	VT (m/s)	n	8	8	9	25
	AT 40	Computed classes				
	Young's modulus	Class	8	8	8	24
	Poisson's ratio	%	100.0	100.0	88.9	96.0
		Predicted classes				
		Class	8	8	8	24
		%	100.0	100.0	88.9	96.0[4]

[1]VT = ultrasound velocity in the transverse direction**[AU18: Please define the AT and FFT terms.]**.

[2]Class = number of cases correctly classified; % = percentage of cases correctly classified.

[3]ST = standard batch; PD1 = defective pressure batch; PD2 = excessive pressure batch; MD = microbial defect batch.

[4]xxx**[AU19: JDS does not permit bold in tables, so bolding has been converted to a footnote. Please define what bolding indicated.]**.

to be the most discriminant variable, although FFT_{75} was also an influential variable (Table 5). For the velocity-related parameters, VL, VT, k, v, and E were also discriminants with a comparatively high weight in the LDA model of the last ripening period (8–10 wk).

The descriptive and predictive performance of the LDA models reflects the reciprocal location of cheese samples in the canonical space of the different ripening periods. In particular, the percentages of the total recognized external samples increased from 70.1 to 80.0% throughout the ripening periods analyzed (from 2–4 wk to 8–10 wk). In general, the correct predictions were relatively lower, between 0 and 65%, for the PD1 cheese samples, mainly due to the misclassification of cheese samples from other batches (PD1 and MD) in the last periods of ripening (6–8 and 8–10 wk; Supplemental Tables S1 and S2, https://doi.org/10.3168/jds .2020-18160). There is high similarity between defects PD1 and MD in soft cheese from the textural viewpoint when the product is at the final stage of ripening. Accordingly, when the LDA models included both defects in the same group (PD1+MD), the percentages of the total external samples recognized were higher than 90% for 6 to 8 and 8 to 10 wk of ripening (Table 6). Figure 4 displays the 40 and 25 cheese samples ripened during 6 to 8 and 8 to 10 wk, respectively, projected in the plane of the 2 DF. The LDA model based on the ultrasonic data of samples with 6 to 8 wk of ripening allowed for differentiation of the 3 groups. Different values of AT 50 and FFT_{75} had a relevant role in the differentiation of PD1+MD and PD2 samples, whereas k and v were the best discriminant parameters for differentiation of ST samples. In samples at 8 to 10 wk, all 3 cheese groups were located in distinct regions of the DF plane. In particular, ST cheese samples were well separated along DF1, whereas DF2 separated PD1+MD samples from PD2 samples by different values of v, AT 40, E, and VT.

CONCLUSIONS

In this paper, we discuss the usefulness of the ultrasound parameters to the quality control of the soft cheese Torta del Casar during ripening, confirming the high correlation between several ultrasonic parameters and both the physicochemical and textural properties of the final cheese product. To the best of our knowledge, in soft cheese, such as Torta del Casar, this is the first literature study to report the discrimination of defective cheeses during ripening by analyzing the ultrasonic parameters and, in turn, validating the on-line capability of ultrasound for nondestructive evaluation of the soft cheese-making process. During the intervals
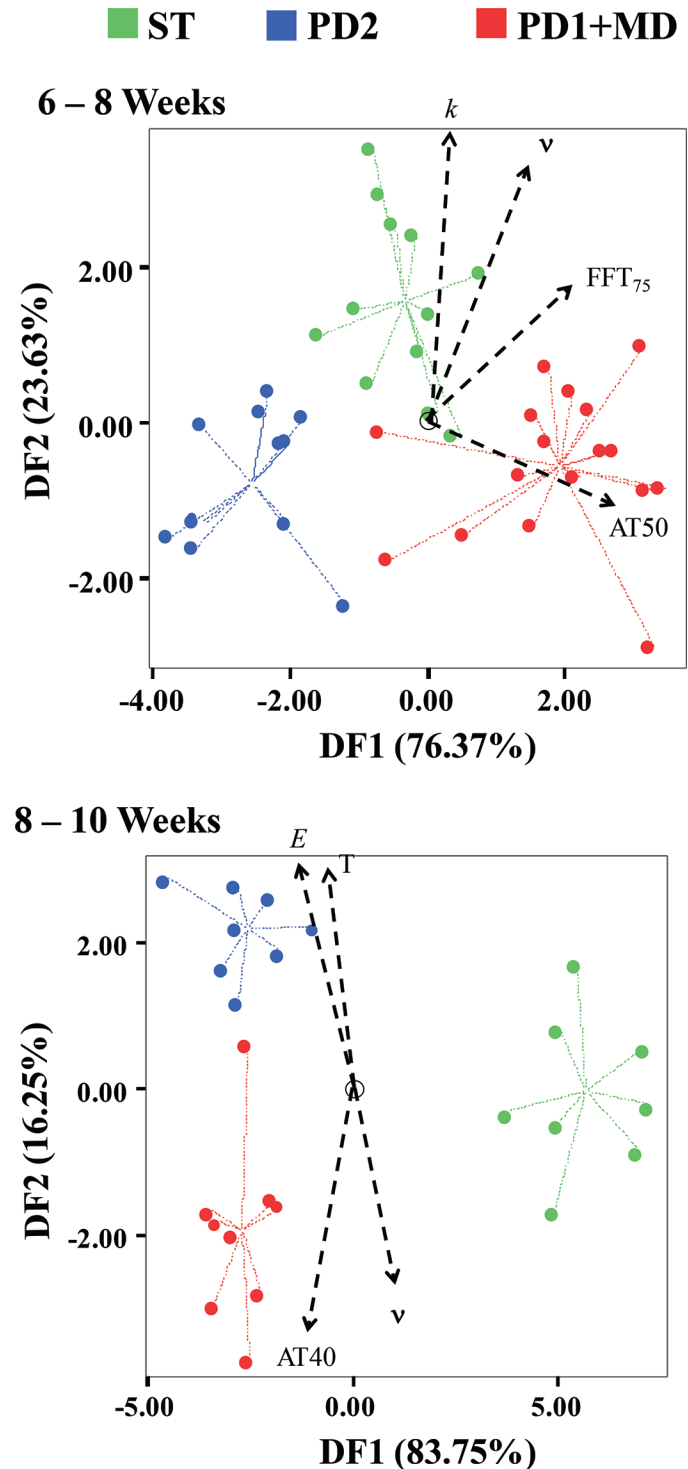

Figure 4. Cheese samples grouped into batches (ST = standard; DP1 = defective pressure; DP2 = excessive pressure; MD = anomalous microbial population) and variable loadings projected on discriminant function (DF) 1 and 2 planes for samples at 6 to 8 and 8 to 10 wk of ripening. k = bulk modulus; v = Poisson's ratio; E = Young's modulus**[AU21: Please define FFT75, AT50, AT40, and T.]**.

of 6 to 8 and 8 to 10 wk of ripening, cheese samples included in PD1 and MD batches differed from the control in the attenuation values (mainly for AT 40 and AT 50). On the contrary, discrimination of cheeses with the PD2 defect (excessive pressure) was mainly based on different values of some velocity-related ultrasound parameters, such as v, k, E, and VT.

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