



Ultrasonic monitoring of softening in solid foods during *in-vitro* gastric digestion

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ABSTRACT

This study aims to evaluate the feasibility of using non-destructive ultrasound to monitor textural softening in potato and cheese during *in vitro* gastric digestion. Textural measurements were taken after different digestion times (5, 10, 15, 20, 25, 30, 60, 90, and 120 min) at 37 °C, while *in vitro* digestion was non-destructively monitored using ultrasound in through-transmission mode.

The textural softening resulted in a substantially greater variation in hardness, ranging from 0.418 to 1.241 N for potato and from 0.200 to 0.534 N for cheese. Meanwhile, the ultrasonic velocity increased during gastric digestion from 745 ± 106.6 m/s to 1342.9 ± 131.5 m/s in potato and from 1377.4 ± 3.8 m/s to 1502.8 ± 4.6 m/s in cheese. Both the softening and velocity increase were attributable to the compositional variation occurring within the food structure due to gastric fluid diffusion into the food matrix. The Weibull model kinetic parameter “k” indicated that potato exhibited a higher softening rate than cheese, due to a faster gastric fluid migration. This was also evidenced by the effective diffusivity of gastric fluid (D_{eff}) obtained by modeling the evolution of the ultrasonic velocity during digestion ($D_{\text{eff, potato}} = 2.5 D_{\text{eff, cheese}}$). A noticeable relationship was found between the softening of both food matrices and the change in the ultrasonic wave velocity. Additionally, it should be highlighted that the measurement of the ultrasonic velocity by ultrasound presented a lower degree of variability than instrumental texture assessment. Therefore, ultrasound proves to be an accurate technique for the on-line monitoring of textural changes in foods during *in vitro* digestion. This non-destructive approach provides a powerful instrumental tool in the design of new products with enhanced nutritional properties by better monitoring how *in vitro* digestion affects their mechanical properties.

1. Introduction

There is growing consumer awareness concerning food composition and processing. The connection between food and health has boosted the development of healthier and nutritionally valuable food products. In response, the food industry is undergoing substantial global transformations and facing new challenges, driven by the need to redesign foods by substituting or altering components and adopting innovative processing technologies (McClements, 2020; Rabadán et al., 2021).

The nutritional properties and functional quality of a food product are not solely determined by its composition and total nutrient content but, more importantly, by the bioavailability of nutrients (Parada and Aguilera, 2007). As such, in the pursuit of novel products aimed at enhancing human well-being, researchers must gain insights into how

new ingredients or modified processing conditions influence the rates of nutrient digestion and absorption (Zhou et al., 2023). Numerous *in vitro* digestion models have been developed to predict nutrient bio-accessibility across various food matrices and products. These models facilitate the exploration of interactions among ingested food components within the human digestive system, thereby contributing to the development of healthier food options that meet consumer demand (Bornhorst et al., 2015; Rodrigues et al., 2022; Sensoy, 2021).

Food digestion begins with mastication in the mouth, which breaks down food into smaller particles. Saliva, containing mucus and amylase, hydrates and lubricates the food. Particularly in the case of starch-based foods, amylase has a significant role in the hydrolysis of starch molecules (Bornhorst, 2017). Peristaltic forces move the food bolus from the esophagus to the stomach. Following the oral phase, the stomach

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becomes the primary site for food degradation within the digestion process, regulating nutrient release into the small intestine (Mackie et al., 2020). Gastric digestion significantly influences nutrient release and bioavailability (Bornhorst and Singh, 2013; Mackie et al., 2013; Mulet-Cabero et al., 2020; Smeets et al., 2021; Bornhorst, 2017) by governing solid food disintegration via chemical degradation caused by the gastric fluid. Gastric fluid, secreted by glands in the stomach lining, contains digestive enzymes, such as pepsin and lipase, as well as hydrochloric acid, altering the food's physical (e.g., texture, such as stiffness or hardness) and chemical (e.g., composition, pH) properties (Kong and Singh, 2009; Kong et al., 2013; Mennah-Govela et al., 2015). Gastric fluid diffusion within the food matrix significantly impacts solid food breakdown and nutrient release during gastric digestion (Mennah-Govela and Bornhorst, 2016a; Nadia et al., 2021), which is influenced by food composition and structure (Drechler and Ferrua 2016; Turgeon and Rioux, 2011; Bornhorst and Singh, 2013, 2014; Sensoy, 2021).

Given the potential impact of the microstructure of processed foods on *in vivo* nutritional responses (Parada and Aguilera, 2007), modifications in structural properties arising from novel ingredient incorporation or adjustments to the processing conditions lead to different behavior during digestion (Parada and Aguilera, 2007; Kong and Singh, 2009; Mackie et al., 2013; Calligaris et al., 2022). A better understanding of the underlying softening mechanism in gastric digestion facilitates accurate predictions of product behavior. This knowledge will be crucial for the development of new food products boasting targeted functional and health properties (Miao and Hamaker, 2021; Gallego et al., 2022). Structural properties are modified during gastric digestion due to the migration of gastric fluids, which induce, among other phenomena, the softening of the solid matrix. These structural modifications will depend on the initial structure and are also time-dependent (Kong and Tran Do, 2022). In order to properly design novel food-structures to improve nutrient bioavailability and adsorption, feasible monitoring techniques to track food structural changes during digestion are necessary (Guo et al., 2020).

Previous literature has highlighted the importance of softening in food breakdown during gastric digestion. Thus, the rate of softening has been proposed as an indicator of the disintegration index for various food matrices (Bornhorst et al., 2015; Do and Kong, 2018; Kong and Singh, 2009; Kong et al., 2013; Kozu et al., 2014; Mennah-Govela and Bornhorst, 2016a). The analysis of softening during *in vitro* digestion is commonly addressed through instrumental textural analysis. The instrumental textural methods employed in the aforementioned studies are, however, time-consuming, destructive, and limited to small sample sizes, highlighting the need in the food industry for a rapid, non-destructive, and accurate system for textural assessment. Numerous non-destructive textural analysis techniques have been explored with the objective of enhancing textural assessment and assessing the feasibility of in-line applications. These techniques can be classified into contact and non-contact types based on the nature of interaction between the sensor and the food material. Some examples of contact techniques are micro-deformation, low impact-based sensors, and acoustic vibrational probes, whereas non-contact techniques involve ultrasonic wave propagation, near-infrared spectroscopy, time-resolved reflectance spectroscopy, hyperspectral imaging, X-ray imaging, and Nuclear Magnetic Resonance spectroscopy (Mishra et al., 2023).

Low-intensity ultrasound has emerged as a promising solution, offering the cost-effective evaluation of textural properties. It has been successfully applied to assess textural properties in a wide range of foods, such as cheeses (Benedito et al., 2000), avocados (Flitsanov et al., 2000; Mizrach & Flitsanov, 1999; Fariñas et al., 2021), potatoes (Sanchez Jimenez et al., 2023), beef steaks (Fariñas et al., 2023), pork fat (Corona et al., 2014), and hams (Corona et al., 2013; Contreras et al., 2020). The interaction of sound waves with matter reveals information about the composition, structure and physical state through changes in velocity and attenuation via absorption and/or scattering mechanisms. Moreover, ultrasound inspection does not require sample preparation,

and its deep penetration capability makes it suitable for monitoring internal quality changes (Khairi et al., 2015).

Therefore, the objective of this study was to gain insight into the use of ultrasound as a non-destructive on-line monitoring technique for predicting changes in food texture during *in vitro* gastric digestion of potato and cheese. The selection of these food samples as reference materials was based on their widespread consumption, nutritional significance, and versatile applicability in various food engineering processes, ensuring a representative comparison within the context of this study (Freitas et al., 2023). Food softening, in turn, noticeably affects its disintegration during digestion and the subsequent release of nutrients from the food matrix. As a result, this research offers an interesting and novel approach that promises to support the design of new or enhanced food structures, with improved functionalities. This will be achieved by establishing meaningful correlations between key parameters governing food digestion, namely textural properties, and ultrasound parameters.

2. Materials and methods

2.1. Chemicals and raw materials

Potatoes and cheese samples were selected as representative foods to investigate the solid food disintegration process during gastric digestion. Raw potatoes (*Solanum tuberosum* var. Soprano) and cheddar cheese were purchased from a local supermarket in Valencia (Spain). The potatoes and cheese were stored at 4 °C and used within 2 days of their purchase. The experimental samples used for *in vitro* digestion consisted of circular slices (25 mm diameter and 10 mm height) (Mennah-Govela and Bornhorst, 2016b; Sanchez Jimenez et al., 2023)

Simulated Gastric Fluid (SGF) was made up of the appropriate electrolyte stock solutions (Brodkorb et al., 2019), pepsin from the porcine gastric mucosa (P7000), CaCl₂, and distilled water. All the chemicals required for *in vitro* digestion were obtained from Sigma-Aldrich.

2.2. Simulated *in vitro* gastric digestion

Despite the key role of the oral stage in particle disintegration during digestion, this analysis focuses exclusively on the gastric phase. Mastication leads to a reduction in the solid particle size, which will affect the characteristic dimension for fluid diffusion during gastric digestion (Jalabert-Malbos et al., 2007) and, consequently, the rate of solid softening (Drechler and Ferrua, 2016). However, molecular diffusion mechanisms are not influenced by particle size. Similarly, particle size does not affect the nature of erosion mechanisms, but obviously changes the contact area between the solid and gastric juice. Therefore, the primary approach in this study is to analyze the diffusion of gastric fluid into the solid particles, which could be considered the most relevant phenomenon involved in the solid disintegration of foods during *in-vitro* gastric digestion under static conditions, and responsible for solid softening. Specifically, the present methodology is driven to establish a preliminary framework for characterizing solid softening by testing the feasibility of a rapid and non-destructive measurement method, namely ultrasound inspection. In our study, the particle size is going to be considered in diffusion mechanisms (see Eq. (7)); thus, the results achieved could be extrapolated to other geometries and sizes in further studies in which the inspection of particles of smaller sizes, as well as creams, purees, and other semi-solid samples, has to be necessarily addressed.

The solid samples were digested following the INFOGEST gastric digestion protocol reported by Brodkorb et al. (2019). Briefly, the SGF was prepared by mixing 75 ml of electrolyte solution with 4.96 ml of water and 20 ml of the pepsin solution, which was prepared by dissolving 1 g of porcine pepsin in 20 ml of distilled water. Finally, the pH of the fluid was adjusted to pH 3.0 using a 1N HCl solution. All the solutions were prepared on a daily basis.

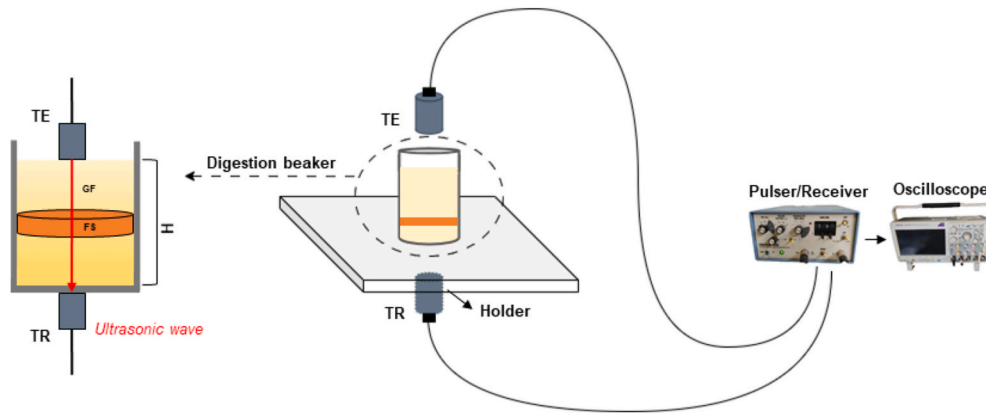


Fig. 1. Ultrasonic set-up (GF: gastric fluid, FS: food sample, TE: emitter transducer, TR: receiver transducer, $H = h_L + L_s$, h_L : gastric fluid height, L_s : sample thickness).

Both the SGF and the samples were tempered in an incubator at 37 °C before initiating the digestion experiments. The digestion experiments were conducted using potato and cheese slices (25 mm diameter, 10 mm height) placed in digestion beakers (radius 26 mm, height 80 mm) made of methacrylate (5 mm wall thickness). These beakers were filled with 100 mL of SGF. The digestion beakers containing the samples were then placed during digestion in a water bath (± 0.1 C; Termotronic-100, P-selecta, Barcelona, Spain) set at 37 °C for 2 h.

For textural analysis purposes, independent digestion trials were performed for each digestion time (5, 10, 15, 20, 25, 30, 60, 90, and 120 min), and each of these trials was replicated three times. In contrast, as the ultrasonic measurement method is non-destructive, the digestion beakers were removed from the water bath for the inspection at the aforementioned digestion times, as explained in section 2.3.2. Once the ultrasonic measurements were completed, in less than 1 min, the digestion beakers were returned to the water bath. Ultrasonic monitoring tests of the digestion were repeated three times for both potato and cheese.

2.3. Food material behavior during simulated gastric digestion

2.3.1. Textural analysis

The textural changes undergone by food samples during gastric digestion were assessed using instrumental textural analysis conducted at 37 °C, immediately after taking the samples from the digestion baker, using a texture analyzer (TA.XT2i, Stable Micro Systems, Surrey, UK), equipped with a 2 mm cylindrical probe (SMS P/2, ANAME, Madrid, Spain). Cheese and potato samples were compressed at 0.5 mm/s test speed up to a strain of 40%. The test was conducted at five points on each slice, using one slice for each replicate ($n = 15$ per digestion time). The experimental data were recorded and processed using Exponent Lite 6.1.4.0 software (Stable Micro System, Surrey, UK). The solid hardness (HA) was computed as the maximum force of compression from the force vs. deformation curve. Changes in the HA (ΔHA_t), in relation to that observed in the fresh product (HA_0), were calculated (Eq. (1)) for each digestion time (t) in order to address the inherent variability of the textural properties in the raw materials. The textural properties of the fresh product were analyzed in each experiment, taking representative samples for each batch of potatoes or cheese ($n = 3$).

$$\Delta HA_t = HA_0 - HA_t \quad (1)$$

where HA_t is the hardness for each digestion time.

The textural changes were described using the Weibull distribution function (Eq. (2)), which has been extensively used to characterize the kinetics of softening in solid foods during *in vitro* gastric digestion (Bornhorst et al., 2015; Drechsler and Bornhorst, 2018; Kong et al., 2013; Somaratne, 2020).

$$\frac{\Delta HA_t}{\Delta HA_0} = \exp(-kt)^\beta \quad (2)$$

where k represents the kinetic parameter (min^{-1}), β is the distribution shape factor (dimensionless) and t the time (min).

2.3.2. Ultrasonic inspection

The experimental set-up, represented in Fig. 1, involved a pair of confronted and well-aligned narrowband transducers (1 MHz, 0.6" diameter, A314S-SU model, Panametrics, Waltham, MA, USA) operating in through-transmission mode. The receiver transducer (TR) was attached to a flat surface, while the emitter transducer (TE) was attached to an automated rod-type actuator with a digital height gauge (192-663-10, Mitutoyo, Tokyo, Japan), connected to a computer, ensuring accurate measurement (± 0.01 mm) of the distance between both TE and TR. For the ultrasonic measurements, coupling gel was used to ensure proper coupling between TR and the base of the digestion beaker. Then, the digestion beaker was positioned over the TR and the TE was moved down until it was immersed approximately 1–2 mm in the gastric fluid, keeping the distance between transducers at a constant 74 mm. Five different measurements were taken in each sample by sliding the digestion beaker over the TR, following a pre-established pattern. It is worth noting that ultrasonic measurements took less than 1 min per sample, ensuring minimal variations in the gastric fluid temperature.

The TE was excited with a 400 V semi-cycle square wave using a pulser/receiver (5077 PR, Olympus, Houston, TX, USA). The ultrasonic signal generated was propagated through the gastric fluid, then through the food sample, and finally through the digestion beaker wall until it reached the TR. The received signal was processed by a built-in low pass filter with a cutoff frequency of 10 MHz and further amplified by -20 and 49 dB for cheese and potato, respectively. Subsequently, the resulting signal was averaged ($n = 128$) and digitized (10 ks at 100 Ms/s) with an oscilloscope (MDO3024, Tektronix, WA, USA) controlled through LabVIEW® (National Instruments, Austin, TX, USA), to be finally stored in a PC. Time-of-flight (TOF) of each signal was computed by the energy threshold method (Garcia-Perez et al., 2019).

Ultrasonic velocity was calculated as the ratio between the propagation path of the ultrasonic wave and its TOF. By acquiring ultrasonic measurements both in the absence and presence of food samples, the ultrasonic velocity through the sample may be computed. For that purpose, the average velocity of the ultrasonic wave in the measurements without (v_{wo}) and with (v_{ws}) sample was estimated using Eqs. (3) and (4), respectively, in which the propagation among the different media is considered.

$$v_{wo} = \frac{h_T}{TOF_{wo}} = \frac{h_T}{h_L/v_L + h_{met}/v_{met}} \quad (3)$$

$$v_{ws} = \frac{h_T}{TOF_s} = \frac{h_T}{h_L/v_L + h_{met}/v_{met} + L_s/v_s} \quad (4)$$

where h_T : distance between transducers, which was kept at 74 mm, h_L : liquid height, h_{met} : methacrylate height (5 mm), L_s : thickness of the sample, v_L : ultrasonic wave velocity in the gastric fluid, v_{met} : ultrasonic wave velocity in the methacrylate, v_s : ultrasonic wave velocity in the food sample. TOF_{wo} and TOF_{ws} represent the time-of-flight of the ultrasonic wave measured without and with the sample, respectively. The value of v_{met} was calculated by measuring the TOF using a disk (30 mm) of this material ($v_{met} = 2648.1 \pm 11.6$ m/s). The ultrasonic velocity through the gastric fluid in the absence of a food sample was calculated from Eq. (3), using the measured TOF_{wo} , resulting in $v_L = 1539.3 \pm 12.3$ m/s. Finally, the ultrasonic velocity through the solid samples was calculated following Eq. (5).

$$v_s = \frac{L_s}{\Delta TOF + L_s/v_L} \quad (5)$$

where ΔTOF represents the TOF difference between TOF_{ws} and TOF_{wo} obtained from the ultrasound signals.

The differences between the ultrasonic velocity in the digested sample at a certain time (v_s) and the velocity in the fresh sample at the initial time (v_0), were computed in order to determine the variations in the ultrasonic velocity (Δv , Eq. (6)) during gastric digestion:

$$\Delta v = v_s - v_0 \quad (6)$$

2.4. Modeling the migration of gastric fluid

For the purposes of gaining a better understanding of the kinetics that determine how the gastric fluid penetrates into the solid food matrix during digestion, both theoretical and empirical models were proposed and their fitting ability was evaluated. The aim of this modeling was to establish a relationship between the rate of gastric fluid migration and the characteristics of the food solid matrix.

2.4.1. Diffusion model

While recognizing the potential coexistence of other mass transport mechanisms, the assumption that the migration of gastric fluid into the solid food is primarily governed by diffusion was adopted. Considering that the size of the slices was adjusted to the internal diameter of the digestion beaker to avoid gastric diffusion in a radial direction, it can be assumed that the samples behave like infinite-slab geometry bodies. Thus, the analytical solution of Fick's second law for the dimensionless content of gastric fluid in an infinite slab was considered (Eq. (7), Crank, 1979). In the present study, we hypothesized that changes in ultrasonic velocity were mainly driven by modifications in the gastric fluid concentration in the samples (Eq. (8)). Based on this approach, the effective diffusivity of the gastric fluid in the solid can be estimated from the variations of the ultrasonic velocity, as illustrated in Eq. (9). Thus, Eq. (9) was fitted to the experimental evolution of the ultrasonic velocity during digestion, with the aim of determining the D_{eff} of the gastric fluid. This model enabled the estimation of the diffusion rate of gastric fluid, considering factors such as negligible shrinkage, uniform initial gastric fluid distribution, and one-dimensional transport.

$$\frac{x_t - x_e}{x_0 - x_e} = \sum_{n=0}^{\infty} \frac{8}{\pi^2(2n+1)^2} \exp(-D_{eff}(2n+1)^2\pi^2t/4L^2) \quad (7)$$

$$\frac{v_t - v_e}{v_0 - v_e} \propto \frac{x_t - x_e}{x_0 - x_e} \quad (8)$$

$$v_t = v_e + (v_0 - v_e) \sum_{n=0}^{\infty} \frac{8}{\pi^2(2n+1)^2} \exp(-D_{eff}(2n+1)^2\pi^2t/4L^2) \quad (9)$$

where v and x represent the average ultrasonic velocity (m/s) and the gastric fluid content (% w.b), respectively. Subscripts t, e, and 0 refer to digestion time (s), equilibrium, and initial conditions ($t = 0$, raw material), respectively. D_{eff} denotes the effective diffusivity of gastric fluid (m^2/s) and L represents the half-thickness of the slice (m). The ultrasonic velocity in equilibrium was also estimated from the fitting of Eq. (9) to the experimental change in the ultrasonic velocity.

2.4.2. Power law model

The assessment of the velocity variation relative to the initial velocity of the fresh samples (Δv) was conducted due to the differences observed among the various samples used in the experimental design, particularly in the case of potatoes. Empirical power law models have previously been employed to describe water and acid migration through different food matrices (Somaratne et al., 2019). Thus, Eq. (10) was used as an alternative model to the diffusional (Eq. (9)) for describing the change in ultrasonic velocity during gastric digestion:

$$\Delta v = Kt^n \quad (10)$$

Where Δv was defined above (Eq. (6) in section 2.3.2), t represents the digestion time (min), k is a kinetic parameter (m/s^{n+1}), and n has been related to the controlling mechanisms of gastric fluid transport, according to Somaratne et al. (2019). For cylindrical shape bodies, values of $n < 0.45$ indicate a low-process rate entirely controlled by diffusion. The increase in n in the range from 0.45 to 0.89 indicates that the control of diffusion is not predominant and other mechanisms related to external transport take relevance. This is the case of surface erosion as a consequence of mechanical force during digestion, which speeds up the overall process by reducing sample size. As for high n values (>0.89), the process approaches a first order-kinetic, which indicates that solid diffusion is no longer relevant and external control plays the key role (Ritger and Peppas, 1987).

2.5. Model fitting and statistical analysis

Regression models were used to establish correlations between the ultrasonic (v , Δv) and textural (ΔHA) parameters and digestion time. The fitting of Weibull (section 2.3.1), diffusion (section 2.4.1), and power law models (section 2.4.2) was performed by minimizing the sum of squared differences between experimental and calculated values. For that purpose, the generalized reduced gradient (GRG) method within the optimization tool SOLVER in Excel™ (Microsoft, WA, USA) was used to identify the model's parameters. The accuracy with which each model fits the experimental data was evaluated by the percentage of explained variance (VAR, %) and the mean relative error (MRE) obtained from the comparison between experimental and simulated data (Dalmau et al., 2017).

Single analyses of variance (ANOVA) ($p < 0.05$) were conducted so as to assess the statistical significance ($p < 0.05$) of the influence of the digestion time (t) on the ultrasonic velocity (v), the variations in ultrasonic velocity (Δv), and hardness (ΔHA). The Fisher's Least Significant Difference (LSD) test with a 95% confidence interval was applied, while the normality of parameters (Δv , ΔHA) was analyzed using the Shapiro-Wilk test. Additionally, linear regression was employed to establish relationships between Δv and ΔHA . The goodness of fit for the model was evaluated by computing the square of the linear regression coefficient (R^2).

All the statistical analyses were conducted using the Statgraphics Centurion XVI statistical package (Statpoint Technologies Inc., Warrenton, VA, USA).

3. Results and discussion

3.1. Texture softening during in-vitro gastric digestion

ΔHA significantly ($p < 0.05$) increased during digestion (Table 1), with a threefold increase from 0.418 to 1.241 N, and from 0.200 to

Table 1
Changes in hardness (ΔHA) during gastric digestion.

Time (min)	Potato		Cheese	
	ΔHA (N)	CV (%)	ΔHA (N)	CV (%)
5	0.418 ± 0.472 ^{c,d}	112.9	0.200 ± 0.076 ^e	38.3
10	0.501 ± 0.495 ^e	99.9	0.256 ± 0.092 ^{d,e}	35.9
15	0.519 ± 0.676 ^{b,c}	130.3	0.309 ± 0.048 ^{c,d}	15.4
20	0.652 ± 0.470 ^c	72.1	0.290 ± 0.070 ^{c,d}	24.1
25	0.882 ± 0.465 ^{c,d}	52.7	0.347 ± 0.078 ^c	22.5
30	0.828 ± 0.475 ^{b,c,d}	57.3	0.358 ± 0.072 ^c	20.1
60	0.992 ± 0.535 ^{b,c}	53.3	0.471 ± 0.052 ^b	11.0
90	1.221 ± 0.740 ^a	60.6	0.559 ± 0.099 ^a	17.7
120	1.241 ± 0.811 ^a	65.3	0.534 ± 0.058 ^{a,b}	11.0

Mean ± standard error. CV: coefficient of variation. Different letters in the same column represent homogeneous groups established from LSD intervals with 95% confidence.

0.534 N for both potato and cheese, respectively. This fact suggests, as illustrated in Eq. (1), that the difference between the hardness of the fresh product (HA_0) and the one for the digested material (HA_t) makes bigger as the *in-vitro* digestion progresses, which is obviously consistent with the expected softening behavior of foods due to the microstructural modifications promoted by acid and enzymatic hydrolysis (Tian et al., 2016, 2017; Kong and Singh, 2008a), which is a fact widely reported in the literature (Mennah-Govela and Bornhorst, 2016a, 2016b). Somaratne (2020) documented 20–40% decrease in sweet potato hardness depending on their specific variety, whereas Do and Kong (2018) documented texture softening of cheddar cheese from 0.96 to 0.20 N during gastric digestion. Despite the agreement with the outcomes of this research, comparing hardness results obtained by different instrumental texture methods can be challenging due to variations in the measurement principles, testing conditions, and equipment used (Drechsler and Bornhorst, 2018) and the lack of standard procedures for food-digestion related studies.

The coefficient of variation (CV) is a dimensionless number that

represents the extent of variability in relation to the mean of a population. It is useful for comparing the precision of laboratory methods. A higher CV indicates greater dispersion and, consequently, lower precision (Reed et al., 2002). As shown in Table 1, higher CV values were obtained for potato, compared to cheese, indicating a greater degree of experimental variability in the measurement of the textural properties, which is also illustrated in Fig. 2.

The softening of cheese and potato during digestion was well described by the Weibull model (Eq. (2)), as illustrated in Fig. 2. High VAR values (94.0% for potato and 97.7% for cheese) and low MRE (7.1% for potato and 4.1% for cheese) were obtained, which demonstrates the accuracy of the Weibull model in describing the textural changes that occur during digestion.

The kinetic parameter of the Weibull model (k) showed that the potato presented a higher rate of softening ($k = 0.0043 \text{ min}^{-1}$) than cheese (0.0005 min^{-1}) (Table 2). The β values for both the potato and cheese were lower than 1, indicating a higher rate of textural softening at the early stage of gastric digestion (Somaratne, 2020). This behavior is coherent with the softening behavior reported for vegetables (Huang and Bourne, 1983; Kong and Singh, 2008b). The disintegration kinetics are greatly influenced by the type of foods which are different in texture and microstructure (Kong & Singh, 2011), such as cheese and potatoes.

Bornhorst et al. (2015) using the rate of softening to propose a food classification system based on the food breakdown observed in several digestion studies. Following this system, food products can be classified according to their material properties and behavior during *in vitro* gastric digestion. Cheese and potato would be classified as two different classes of food products based on their initial hardness and rate of softening, along with the proposed rate-limiting breakdown mechanisms. Thus, in the case of potatoes, the softening rate depends on the diffusion rate of gastric juice, which will be dependent on the initial structure of the raw material and the microstructural modifications undergone during gastric digestion (Mennah-Govela and Bornhorst, 2016a; Kong et al., 2011). Potatoes have a high content of water (~80%), with pectin (~55%) and cellulose (~28%) as the main cell

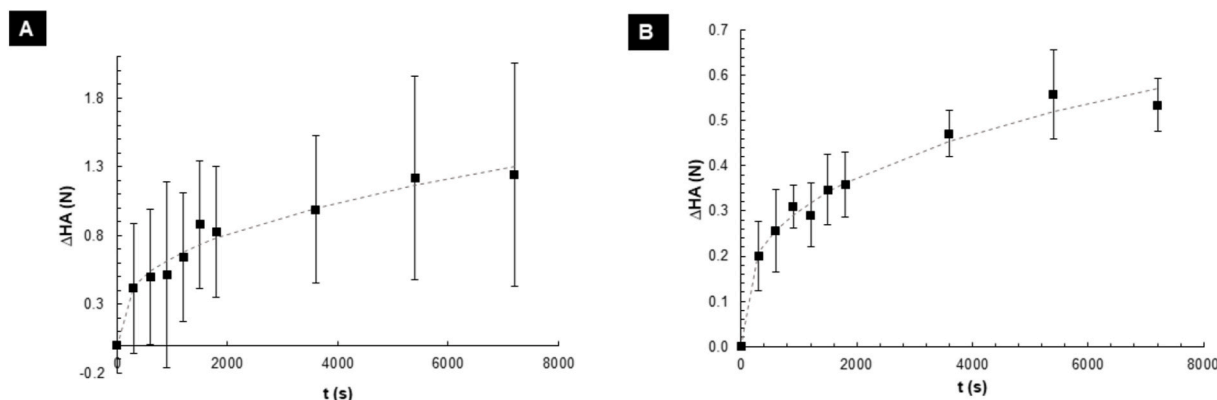


Fig. 2. Evolution of the variation in hardness (ΔHA) for potato (A) and cheese (B) slices during gastric digestion. Each point represents the mean value of the experimental measurements and dotted grey lines correspond to the Weibull model. Error bars represent the standard deviation.

Table 2
Parameters and statistical coefficients of Weibull (Eq. (2)), diffusion (Eq. (9)), and power law models (Eq. (10)).

	Model equation						
	2	9	10				
Model parameters	k (min^{-1})	β	D_{eff} (m^2/s)	v_e (m/s)	k ($\text{m}/\text{s}^{(n+1)}$)	n	
	Potato	0.0043	0.246	5.3×10^{-9}	1334.6	106.4	0.37
	Cheese	0.0005	0.274	2.1×10^{-9}	1553.0	3.5	0.77
Statistical coefficients	VAR (%)	MRE (%)	VAR (%)	MRE (%)	VAR (%)	MRE (%)	
	Potato	94.0	7.1	99.1	0.8	96.7	5.6
	Cheese	97.7	4.1	91.1	1.2	97.6	9.6

D_{eff} : effective diffusivity of gastric fluid, v_e : equilibrium ultrasonic velocity, VAR: explained variance, MRE: mean relative error.

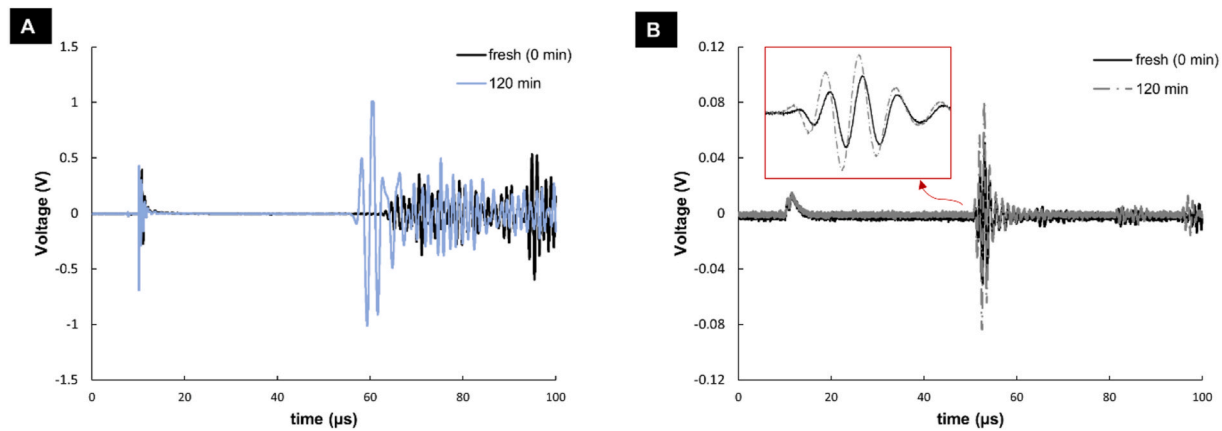


Fig. 3. Ultrasonic signals propagated through the digestion baker with slices of potato (A) and cheese (B) at the initial time ($t = 0$, fresh samples) and after 120 min of gastric digestion.

components, where the structure and organization of pectic polysaccharides play a crucial role in determining cell wall permeability (Ding et al., 2021). The textural changes in these foods during digestion are mainly linked to the degradation of cell wall and middle lamella structural components, as reported by Mennah-Govela and Bornhorst (2016a) from microstructural analysis. As gastric digestion progresses, the cell arrangement that forms the microstructure of potatoes may be disrupted, leading to the loss of cell wall integrity and firmness due to gastric fluid absorption resulting in the tissue softening. Mennah-Govela and Bornhorst (2016a) reported a cell wall breakdown in steamed sweet potatoes after 240 min of digestion, indicating the capability of gastric acid to break down sweet potato cell walls.

Jiménez-Munoz et al. (2022) studied how the structure of a potato protein affects the diffusion of gastric juice. This behavior was also observed in the case of the gastric digestion of carrots (Kong and Singh, 2009, 2011), where the textural softening observed during gastric digestion was mainly caused by the acidic and enzymatic hydrolysis of the pectin material, resulting in cell separation and cell wall damage. Somaratne (2020) reported similar results when studying the microstructural evolution of different kinds of potato during gastric digestion by light microscopy.

In the case of cheese, which is considered as a protein gel containing fat globules, water, and other materials (Guo et al., 2020), its softening will be mainly induced by the rate at which the protein matrix structure is weakened by the acid and enzymes contained in the gastric fluid. Do and Kong (2018) reported the occurrence of an extensive protein network degradation during the *in vitro* digestion process of Cheddar cheese, due to protein hydrolysis and the formation of structurally weaker structures via the disruption of fat globules. Similarly, Fang et al. (2016) investigated the *in vitro* digestion of cheeses with different fat

contents, such as Camembert, Mozzarella, and Cheddar, and reported the impact of the protein-to-fat ratio in the rate of disintegration. The release of fat during digestion may affect the cheese texture and, consequently, its disintegration rate. A high protein content will lead to a denser packed structure, whereas fat softens the cheese texture. Additionally, specific dairy structures can lead to a different behaviour in the gastric phase. Mulet-Cabero et al., 2017 studied two dairy-based foods with the same fat content but different structures. These authors concluded that different food structures can significantly alter gastric digestion, even when the fat content is the same. Kong and Singh (2008b) examined the texture of carrot and ham samples, chosen as representative foods, for the purposes of investigating the solid food disintegration process during gastric digestion. They hypothesized that the difference in the disintegration behavior of these samples was related to the competition between surface erosion and textural softening. The authors identified two competing mechanisms controlling the breakdown of the solid foods: surface erosion caused by gastric forces, and tenderization (textural softening) via gastric fluid action. However, in our study, as stomach-generated mechanical forces are not present, only the softening mechanism resulting from the gastric fluid action contributes to the textural modifications in the food matrix.

3.2. Modifications of ultrasound parameters during *in-vitro* gastric digestion

Fig. 3 shows the ultrasonic signals transmitted through the digestion beaker for potato (A) and cheese (B) at the initial time (raw sample) and at the end of digestion. It is observed that the TOF at 120 min is shorter than at the beginning of digestion, which is especially noticeable for the potato experiments. This demonstrates that the modification of the

Table 3

Ultrasonic velocity (v) and changes in ultrasonic velocity (Δv) in potato and cheese during *in-vitro* digestion.

Time (min)	Potato			Cheese		
	v (m/s)	CV (%)	Δv (m/s)	v (m/s)	CV (%)	Δv (m/s)
0	745.3 ± 105.6^e	14.2	0.0 ± 0.0^e	1377.4 ± 3.8^e	0.3	0.0 ± 0.0^f
5	$888.3 \pm 44.3^{d,e}$	5.0	$143.1 \pm 13.7^{3d,e}$	$1387.7 \pm 6.5^{e,f}$	0.5	10.3 ± 10.2^f
10	$970.3 \pm 103.5^{c,d}$	10.7	$225.0 \pm 174.1^{d,e}$	$1393.8 \pm 5.3^{e,f}$	0.4	$16.4 \pm 9.1^{e,f}$
15	$1013.2 \pm 110.9^{c,d}$	11.0	$267.9 \pm 185.3^{c,d,e}$	$1396.6 \pm 9.8^{d,e,f}$	0.7	$19.2 \pm 13.6^{d,e,f}$
20	$1103.0 \pm 111.8^{b,c}$	10.1	$357.7 \pm 158.1^{b,c,d}$	$1411.2 \pm 9.2^{c,d,e}$	0.6	$33.8 \pm 5.4^{c,d,e}$
25	$1116.2 \pm 62.9^{b,c}$	5.6	$370.9 \pm 118.9^{b,c,d}$	$1419.1 \pm 15.9^{c,d}$	1.1	$41.7 \pm 12.1^{c,d}$
30	$1145.7 \pm 79.8^{b,c}$	7.0	$400.4 \pm 139.1^{b,c,d}$	1425.2 ± 13.9^c	1.0	47.8 ± 10.1^c
60	$1260.7 \pm 156.2^{a,b}$	12.4	$515.4 \pm 206.6^{a,b}$	1462.4 ± 23.0^b	1.6	85.0 ± 19.2^b
90	$1280.2 \pm 153.9^{a,b}$	9.8	$534.9 \pm 198.5^{a,b}$	1497.5 ± 0.1^a	0.1	120.1 ± 3.9^a
120	1342.9 ± 131.5^a	18.6	597.6 ± 181.6^a	1502.8 ± 4.6^a	0.3	125.4 ± 8.4^a

Mean \pm standard error. CV: coefficient of variation. Different letters in the same column represent homogeneous groups established from LSD intervals with 95% confidence.

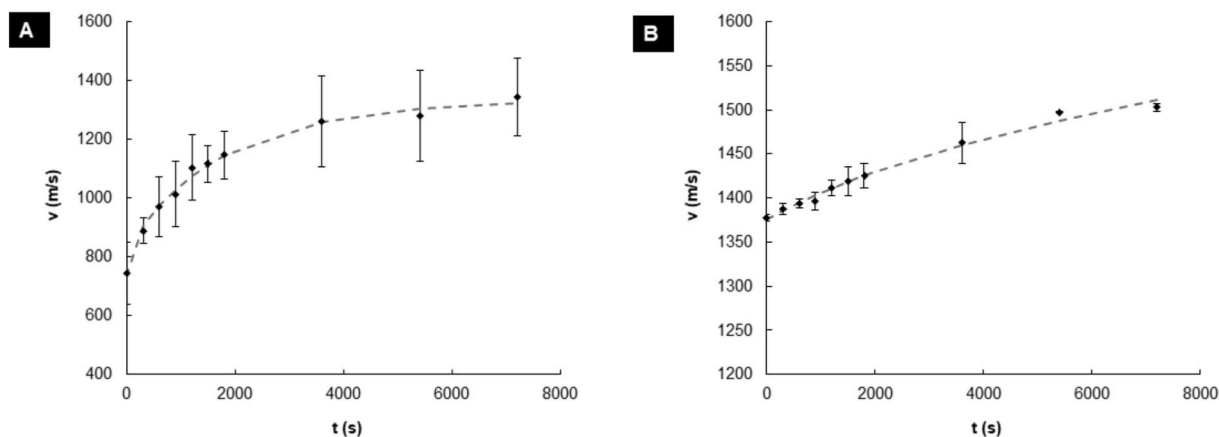


Fig. 4. Change of the ultrasonic velocity (v) in potato (A) and cheese (B) slices during gastric digestion. Each point represents the mean value of the experimental measurements and dotted grey lines correspond to the Diffusion model. Error bars represent the standard deviation.

ultrasonic wave velocity was more pronounced for potato than cheese. This may be explained by the higher softening rate of potato, as reported in the previous section (Tables 1 and 2), which could be ascribed to its own structure as well as to a faster migration of the gastric fluid.

The sample size remained constant during the digestion process. Thus, considering that velocity is defined as the thickness of the slice through which the wave is propagated, divided by the TOF, a shortening in TOF results in an increase in velocity. The evolution of ultrasonic velocity (v) and the change in velocity (Δv) are shown in Table 3.

The ultrasonic velocity of the fresh samples was 745.3 ± 105.6 m/s and 1377.4 ± 3.8 m/s for potato and cheese, respectively. These values coincide with those previously reported for these types of products (Benedito et al., 2000; Sanchez-Jimenez et al., 2023). The differences observed between the ultrasonic velocities of potato and cheese are attributed to the distinct structural properties inherent to these two food matrices. The structure of potato is characterized by a large porous network (Somaratne, 2020), with its porosity, and the associated air content, which is higher than that of the cheese matrix. This difference in porosity may be responsible for the lower ultrasonic velocity observed in potato compared to cheese, which consists of a protein-gel network. In the case of potatoes, Sanchez-Jimenez et al. (2023) reported an ultrasonic velocity of 509 ± 5 m/s, while Ha et al. (1991) found a value of 824 m/s. These authors studied different varieties of potatoes, which could explain the difference between the figures of the ultrasonic velocity. Moreover, both previous studies highlighted the high degree of experimental variability of the ultrasonic velocities in potato, which was also evidenced in the present analysis (Table 3), in which the CV was 14.2%.

As for the cheese, Benedito et al. (2000) found an ultrasonic velocity of 1585 m/s in cheddar cheese, evaluated at a temperature of 35 °C. The difference in the ultrasonic velocity reported by Benedito et al. (2000) and that found in the present study at 37 °C (1377.4 m/s) could be linked to variations in the fat content, as well as to the percentage of melted fat.

A lower degree of variability was found in the experimental determination of the ultrasonic velocity in cheese ($CV < 1.6\%$), compared to that of potato ($CV < 18.6\%$) (Table 3). This finding was expected since, as already shown by the textural measurements, potato presented a greater experimental variability than cheese in terms of its mechanical properties. In addition, fruits and vegetables are recognized as highly attenuating materials, due to their porous structure, which produces the scattering of elastic waves and, therefore, increases the variability in the measurement of the ultrasound parameters (Mizrach, 2008).

The changes in ultrasonic velocity were statistically significant ($p < 0.05$) throughout the digestion process, evidencing an increase from 745.3 to 1342.9 m/s for potato and from 1377.4 to 1502.8 m/s for

cheese, as shown in Table 3. With the progression of digestion, the gastric fluid penetrates into the potato and cheese slices, thereby inducing the softening of the food matrix, a well-documented phenomenon (Kong and Singh, 2009; Mennah-Govela and Bornhorst, 2016a; Sensoy, 2021). Therefore, a greater degree of softening would be expected to induce a decrease in the ultrasonic velocity, since acoustic waves are greatly affected by the mechanical properties of the propagation medium (Benedito et al., 2000; Corona et al., 2013). However, in the present study, we have observed that the ultrasonic velocity increases as the sample softens and hardness decreases. This phenomenon can only be explained by the fact that textural changes, related to structural changes in the food matrix, are accompanied by a change in the food composition, due to the migration of the gastric fluid into the matrix. This change in composition also affects the ultrasound wave propagation through the food matrix and, consequently, the ultrasonic velocity is altered. In the case of potato, part of the air occupying the inner and outer cell spaces are replaced by the gastric juice, which increases the ultrasonic velocity, since in the gastric fluid ultrasound propagation is much faster than in air. Meanwhile, in the case of cheese, the compositional variation is attributed to the increase in the aqueous content, due to gastric fluid diffusion, which reduces the fatty fraction. In this regard, it is important to highlight that, the ultrasonic velocity in fat is lower at 37 °C than in the gastric fluid aqueous solution (Benedito et al., 2000; Telis-Romero et al., 2011), which leads to an increase in the ultrasonic velocity in the cheese matrix. Therefore, the change in ultrasonic velocity during digestion is more affected by the compositional variation in the food sample than by the textural modifications, although both phenomena are closely linked since they are related to the diffusion of gastric fluid through the food matrix. These results strongly support our initial hypothesis, indicating that ultrasonic velocity changes are directly related to the diffusion of the gastric fluid into the food matrices, as stated in Eq. (8).

The tendency to increase exhibited by Δv was similar to that of velocity as digestion progresses for both potato and cheese. The velocity increase observed in potato (80.2%) was substantially greater than that in cheese (9.1%). The smaller increase in velocity through the cheese matrix could be attributed to the similarity of the ultrasound velocity in the raw cheese and the gastric fluid (1377.4 vs. 1539.3 m/s, respectively), while in the case of potatoes, the differences are much larger (745.3 vs. 1539.3 m/s).

3.2.1. Modeling the migration of gastric fluid into the food matrix from the ultrasonic velocity

Results from the textural analysis indicated that the *in vitro* digestion process resulted in the softening of solid foods, which is attributed to the migration of gastric fluid within the food matrix. Furthermore,

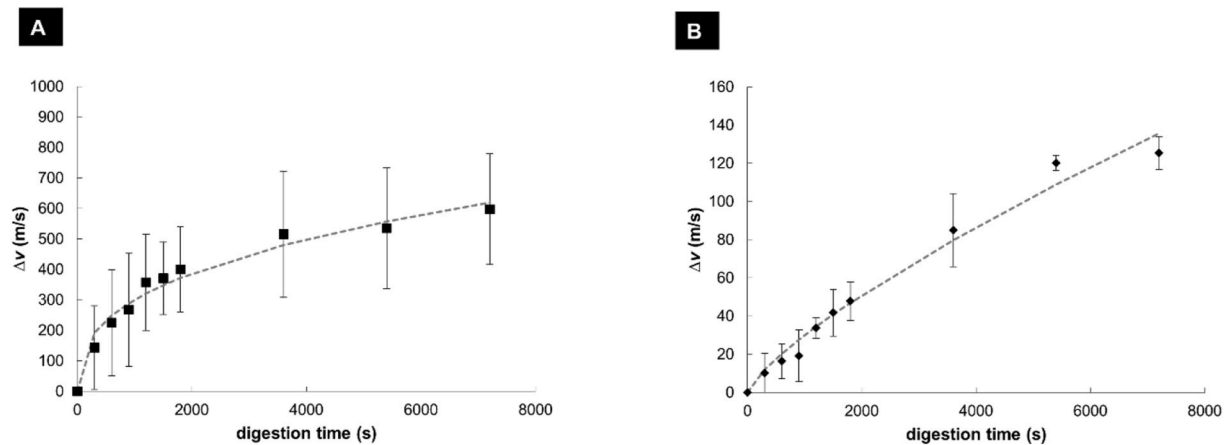


Fig. 5. Evolution of the ultrasonic velocity variation (Δv) in potato (A) and cheese (B) slices during gastric digestion. Each point represents the mean value of the experimental measurements and dotted grey lines correspond to the power law model. Error bars represent the standard deviation.

discernible differences in this softening behavior were observed between potato and cheese samples. Ultrasound inspection also revealed disparities in the changes of ultrasonic velocity during digestion. Thus, it becomes imperative to gain a comprehensive understanding of the mechanisms that govern the migration of gastric fluid within solid foods. To better understand how quickly gastric fluid permeates into the solid food matrix, two mathematical models were proposed to test the feasibility of estimating gastric fluid diffusion from the ultrasonic velocity. This approach is based on the idea that the diffusion of gastric fluid is the main phenomenon involved in the change in the ultrasonic velocity, as shown in section 3.2. Based on this assumption, the effective diffusivity, as well as other kinetic parameters, of gastric fluid migration into the solid food could be estimated from the ultrasonic velocity. Thus, the observed increase in the experimental v and Δv during gastric digestion were modeled using the diffusion approach (Eq. (9)) and the power law function (Eq. (10)), respectively.

As illustrated in Fig. 4, the diffusion model was an adequate fit for the evolution of the ultrasonic velocity in potato and cheese matrices during gastric digestion. The statistical parameters (Table 2) reflected the accuracy of the modeling, since VAR reached values of 99.1% and 91.1% for potato and cheese, respectively, while the MRE values were 0.8% (potato) and 1.2% (cheese). These results highlighted that the evolution in the ultrasonic velocity follows a pseudo diffusion pattern due to its proportionality to the change in the gastric fluid concentration in the solid matrix. In the case of cheese, the low VAR values obtained suggest that another mechanism could take relevance in gastric fluid migration, such as external convective transport due to the fact that the experiments were carried out in static conditions.

Based on the above-mentioned approach, the estimated effective diffusivities (D_{eff}) were 5.3×10^{-9} and 2.1×10^{-9} m^2/s for the diffusion of the gastric fluid through potato and cheese, respectively. Mennah-Govela and Bornhorst (2016a) reported a very similar gastric fluid D_{eff} (1.6×10^{-9} m^2/s) in their research into the gastric digestion of sweet potatoes. Similarly, Widjaja (2010) found that the effective diffusion coefficient of gastric fluid in potatoes changed over the digestion time, shifting from 4×10^{-10} m^2/s at 1h to 1.3×10^{-9} m^2/s at 3–4 h. Meanwhile, the literature documenting the diffusion of small molecules within cheese matrices, with the exception of salt and moisture, is notably limited (Floury et al., 2010). Simal et al. (2001) reported diffusion coefficients of NaCl and water of 5.3×10^{-10} m^2/s and 7.8×10^{-12} m^2/s , respectively, in Mahon cheese. The diffusion of gastric fluid is governed primarily by the gradients of water and acid concentration existing between the food matrix and the gastric fluid, complemented by the migration of electrolyte ions, such as Cl and HCO_3Na , among others (Sehgal et al., 2022). Therefore, the D_{eff} figure estimated in our study is consistent with those reported in the literature.

The D_{eff} found in the potato matrix was approximately 2.5 times higher than that of cheese, indicating a higher gastric fluid diffusion rate in potatoes. This finding is coherent with the higher rate of softening, as previously described (section 3.1). The differences between the two types of foods could be ascribed to the more porous structure network (that can be filled with air or with an aqueous solution) of the potato matrix when compared to that of cheese. Upon immersion of the food slice into the gastric fluid, the liquid permeates into the food matrix, moving towards the center of the sample, as previously reported by Somaratne et al. (2019). These authors suggested that a less porous and more rigid structure might hinder the mobility of gastric fluid within the food matrix. Thus, it can be inferred that the greater gastric fluid diffusivity within potatoes is related to the interconnected porous network that constitutes their microstructure (Sanchez Jimenez et al., 2023), as opposed to the denser cellular microstructure of cheddar cheese, which could hinder the path of gastric fluid diffusion. In the case of cheese, the ultrasonic velocity in equilibrium (v_e) was observed to closely match that of gastric fluid (1553 m/s vs. 1539 m/s), while the potato had a lower v_e value (1334.6 m/s). The equilibrium figure for the ultrasonic velocity in both food materials is not only affected by the exchange of fluids but also by its own structural properties.

The experimental data were also modeled using a power law function in order to verify the gastric fluid transport mechanism within the food matrices. Table 2 presents the parameters of the proposed model (k , n). Fig. 5A and B illustrate the curves of the experimental and predicted values for both potato and cheese samples, respectively. The figures of the VAR (96.7% and 97.6%, for potato and cheese, respectively) and MRE (potato: 5.6%, cheese: 9.6%) reflected a high degree of accuracy of the power law model to describe the changes in Δv during gastric digestion in both food matrices. As can be seen in Table 2, a lower k value was found for the cheese matrix ($k = 3.5$ $\text{m}/\text{s}^{(n+1)}$), compared to the potato samples ($k = 106.4$ $\text{m}/\text{s}^{(n+1)}$), evidencing a slower kinetic. This result is coherent with the findings from the diffusion approach (D_{eff} : cheese < potato) and the textural measurements (i.e., lower softening rate for cheese than for potato). The exponent n parameter was found to be lower than 0.45 for potatoes, indicating that the migration of gastric fluid into this food matrix followed a pure diffusion pattern. Conversely, for cheese, it was found that n was between 0.45 and 0.89, suggesting that external transport mechanisms would also play a noticeable role in the gastric fluid migration. This aligns with the fitting of the diffusion model in cheese, which exhibited a lower degree of accuracy compared to that in potato.

3.3. Monitoring textural changes using ultrasound

As shown in Fig. 6, a close relationship was observed between $\Delta \Delta A$

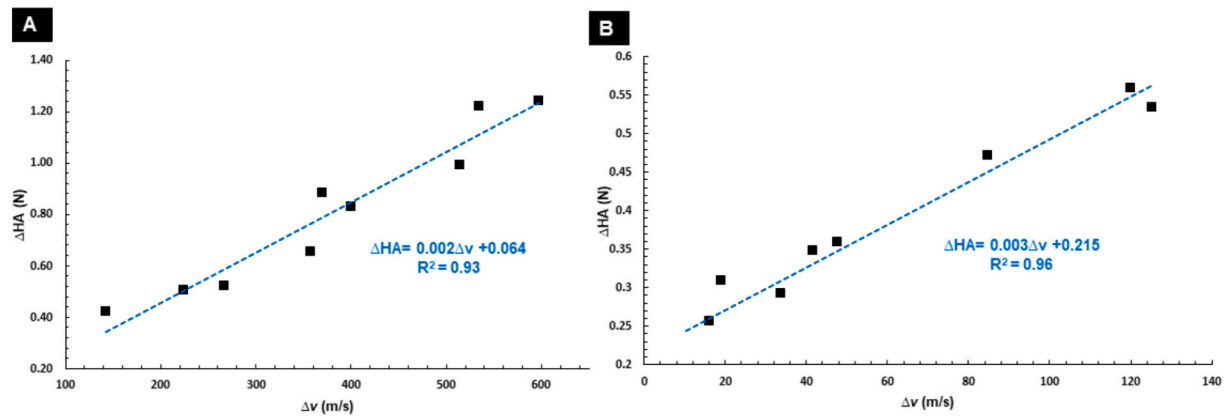


Fig. 6. Relationship between the variation in ultrasonic velocity (Δv) and the change in hardness (ΔHA) for the potato (A) and cheese (B) slices during gastric digestion. Each point represents the mean value of the experimental measurements and dotted blue lines correspond to the linear fitting.

and Δv for both potato and cheese samples during digestion ($R^2 > 0.93$). The softening of the food matrix, as described by ΔHA , was found to be associated with the increase in ultrasonic velocity. Likewise, the correlation between ultrasonic velocity and textural properties has been explored previously. For example, [Corona et al. \(2013\)](#) noted that an increase in the hardness of lean and fatty tissue in dry-cured ham led to a rise in ultrasonic velocity. Similarly, [Contreras et al. \(2020\)](#) demonstrated the feasibility of using ultrasound as a non-destructive technique for evaluating the pastiness of dry-cured ham. [Benedito et al. \(2000\)](#) reported a linear relationship between the square root of the deformability modulus and ultrasonic velocity, showing an increase in the modulus as the velocity increased during cheddar cheese maturation. However, the trend found in this study went against what was expected, as mentioned in section 3.2. This discrepancy comes from the fact that, in the present analysis, the change in ultrasonic velocity was mainly attributed to variations in the composition of the food matrices. This composition is altered by the migration of the gastric fluid into the structure as digestion progresses, resulting in an increase in velocity and the simultaneous softening of the solid matrix. Therefore, as changes in composition and texture take place simultaneously, the increase in velocity during gastric digestion is indirectly related to the decrease in texture, and therefore, the non-destructive measurement of the ultrasonic velocity could serve as a means both to assess the textural changes experienced by foods during *in vitro* digestion and to monitor gastric juice migration. This could potentially facilitate the online monitoring of the digestion process through an innovative approach, namely ultrasound, which has several advantages. These include a non-destructive nature, rapid and cost-effective characteristics, and the versatility necessary to examine a wide variety of geometries and sizes. Additionally, ultrasonic measurements provide a reduced variability compared to that obtained by traditional instrumental textural analysis, the CV values for textural measurements being approximately one order of magnitude greater than those obtained for the ultrasonic velocity (see [Tables 1 and 3](#)).

The indirect relationship between velocity and textural properties identified in this research holds significance for the field of food development, as it could help in the design of new products with improved nutritional properties by easily monitoring their textural changes during gastric digestion, which can be assessed rapidly, accurately, and non-destructively. Exploring various food products with diverse physical properties, including solids and semi-solids, could be a topic for future research so as to determine if this technique is applicable to other food types. Furthermore, future studies should explore the textural changes that may occur in real-time situations, considering factors such as peristaltic movement and mastication, to provide a more comprehensive understanding of the dynamic nature of food texture during digestion.

4. Conclusions

This study has demonstrated the feasibility of using ultrasound to monitor the gastric fluid migration and textural softening of potato and cheese matrices during gastric digestion. The observed increase in the ultrasonic velocity during digestion can be attributed to the migration of gastric fluid into the food matrices, which directly controls the softening of the food structure. The diffusion model was used to mathematically describe the changes in ultrasonic velocity in the food matrices during *in-vitro* digestion. The fitting of the diffusion model revealed that gastric fluid migration was faster in potato than in cheese matrices, which was consistent with the observed softening rate. Moreover, the change in the ultrasonic velocity and the hardness were found to be closely related. In addition, the ultrasonic testing showed a much lower degree of experimental variability than that found in instrumental textural assessment.

This research highlights the potential of ultrasound as a valuable tool for monitoring gastric fluid migration and the textural changes in foods during *in vitro* digestion. This non-destructive approach has the potential to facilitate the development of new products with optimized textural properties, ultimately contributing to the design of healthier foods with improved nutritional properties. Future studies exploring a variety of food types with diverse mechanical properties could further expand the application of this innovative technique.

CRedit authorship contribution statement

Anabella S. Giacomozzi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **José Benedito:** Writing – review & editing, Funding acquisition. **Amparo Quiles:** Writing – review & editing. **José V. García-Pérez:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **María Esperanza Dalmau:** Writing – review & editing, Supervision, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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