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Characterization of the mechanical properties of high-moisture meat analogues using low-intensity ultrasound: Linking mechanical properties to textural and nutritional quality attributes

R.-M. Guillermic [a,c], A.J. Franczyk [a], S.O. Kerhervé [b], J.D. House [a], J.H. Page [b], F. Koksel [a,*]

[a] Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada
[b] Department of Physics and Astronomy, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada
[c] Département de Sciences Mathématiques et Physique, Université de Saint-Boniface, Winnipeg, Manitoba R2H 0H7, Canada

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ABSTRACT

Plant-based meat analogues offer possible alternatives to meat consumption. However, many challenges remain to produce a palatable meat analogue as well as to understand the roles of different processing steps and ingredients on both the texture and nutritional properties of the final product. The goal of this paper is to help with addressing these challenges by using a low-intensity ultrasonic transmission technique, both online and 24 h after production, to investigate high-moisture meat analogues made from a blend of soy and wheat proteins. To understand the ultrasonic data in the context of traditional characterization methods, physical properties (meat analogue thickness, density, peak cutting force) and protein nutritional quality attributes of the meat analogues were also characterized separately. The ultrasonic velocity was found to decrease with the feed moisture content and to be strongly correlated ($r = 0.97$) with peak cutting force. This strong correlation extends over a wide range of moisture contents from 58% to 70%, with the velocity decreasing from about 1730 m/s to 1660 m/s over this range. The protein quality was high for all moistures, with the highest amino acid score and *in vitro* protein digestibility being observed for the highest moisture content treatment. The accuracy of the ultrasonic measurements was enhanced by the development of an innovative non-contact method, suitable for materials exhibiting low ultrasonic attenuation, to measure the meat analogue thickness ultrasonically and in a sanitary fashion – an advance that is potentially useful for online monitoring of production problems (e.g., extruder barrel-fill and cooling-die temperature issues). This study demonstrates, for the first time, the feasibility of using ultrasonic transmission techniques to measure both velocity and sample thickness simultaneously and provide information in real time during production that is well correlated with some textural and nutritional attributes of meat analogues.

1. Introduction

In the context of climate change and increased need for finding alternative ways to feed the growing world population, plant-based meat analogues have become an interesting route for food innovation. As customers look more and more for protein alternatives, the global plant protein market is expected to grow further, attracting investors and changing the agribusiness landscape progressively in North America and around the globe. However, using plant-based proteins to produce food with fibrous and/or layered texture and nutritional quality similar to meat remains challenging. Different routes to improve this type of product have been taken, such as work on the processing side (by improving typical extrusion processing conditions or creating new processing techniques such as the shear cell), or the composition side (by finding innovative plant-based ingredients capable of being texturized, and possessing an appreciated taste and good nutritional quality).

To form a product with elongated muscle-like fibers, the most widely preferred processing technology is extrusion cooking, but some other recent innovations suggest the use of a shear cell (Cornet, et al., 2022), or even 3D food printing (Ramachandraiah, 2021). The advantage of extrusion cooking relies on its continuous operation, making it a very good candidate for large scale production. However, the process is very complex and involves many interacting parameters: pressure build-up towards the die, temperature profiles across the barrel and the die,

* Corresponding author.
E-mail address: Filiz.Koksel@umanitoba.ca (F. Koksel).

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shear forces, and cooling die dimensions to cite only a few. It is therefore extremely challenging to pinpoint the exact set of parameters that would make the process reproducible from one extruder to the next, especially for those with different capacities (e.g., bench-top, pilot or industrial scales). It is also equally challenging to pinpoint the exact characteristics that would make the "perfect" high-moisture meat analogue. The fibrous structure of meat analogues can vary with the ingredients and the set of processing conditions used and so does the texture (Schreuders, et al., 2021) and the nutritional quality (Alessandrini, et al., 2021). In this context, it would be extremely interesting to develop a tool that can help tackle these issues by providing a way to understand the link between the composition, processing and final product mechanical and nutritional properties. Providing a quality control tool able to distinguish between different process conditions, and thus different meat analogues, in real time would also be a real asset for this emerging industry. Variations in the properties of meat analogues during continuous manufacturing operations, either by a temporary instability or a production-line problem, pose significant challenges to the industry. Accordingly, being able to detect changes in the quality of meat analogues in real time could also ensure less downtime in the production line and less waste. Also, assessing the thickness of meat analogues during production would be a great advantage because a change in meat analogue thickness can indicate, for example, issues related to extruder barrel fill or fluctuations in the cooling die temperature.

To propose a potential solution to some of these challenges, this paper presents a method, using air-coupled low-intensity ultrasound, to measure the ultrasonic properties of meat analogues during and after production. The rationale for this approach is the fact that the use of low-intensity ultrasound in the food industry in the context of non-destructive process control and product assessment has 25 + years of history. These applications include the use of ultrasonics for the measurement of liquid fill level in a tank, detection of bottles flowing in a production line, measurement of sugar content in a solution, etc. (Mulet, Benedito, Golàs, & Càrcel, 2002). Recent studies on more complex food systems have also shown the potential of low-intensity ultrasonics as a powerful tool to assess the presence of bubbles and measure the evolution of the bubble size distribution in bread dough (Koksel, Scanlon, & Page, 2016). Furthermore, ultrasonic methods have also proved useful in real time during noodle dough production (Kerhervé, et al., 2019) to detect thickness fluctuations in production and the effect of composition (water and amount of salt) on dough properties.

In this study, a non-destructive non-contact on-line quality control tool utilizing low-intensity ultrasound waves is developed and applied for the first time on a food extruder to measure the mechanical properties of high-moisture meat analogues during production. The aim of this research is to correlate the ultrasonic signature of the meat analogues (measured in real time during production and 24 h after) with its textural (i.e., longitudinal and transverse cutting force, degree of texturization) and nutritional (i.e., amino acid profile, amino acid score, *in vitro* protein digestibility and *in vitro* protein digestibility corrected amino acid score) attributes (measured separately in an off-line manner). We show that the ultrasonic signal can also be used to access the thickness of meat analogues without contact, and we present the advantages, drawbacks and precautions that should be taken into account to up-scale this method.

2. Materials and methods

2.1. Raw material characterization

Soy protein concentrate (Solcon F) and soy protein isolate (Solpro 910) were purchased from Solbar (Ningbo, Zhejiang, China), while wheat gluten was kindly provided by Archer Daniels Midland Agri-Industries Company (Montreal, QC, Canada). The protein content, as measured according to the AACC International method 46–13 (AACC, 1999), of the soy protein concentrate (SPC), soy protein isolate (SPI) and vital wheat gluten was 65.7, 87.5 and 85.2% dry basis (db), respectively. Based on previous literature (Kim, Riaz, Awika, & Teferra, 2021), SPC, SPI and vital wheat gluten were mixed to achieve a final protein content of ~ 76% with the following composition 58% SPC, 27% SPI and 15% vital wheat gluten.

2.2. Extrusion cooking of high-moisture meat analogues

Extrusion cooking of high-moisture meat analogues was performed in triplicate with a twin-screw extruder (MPF19, APV Baker Ltd., Peterborough, UK) with a 19 mm screw diameter and a 25:1 barrel length-to-screw diameter ratio, using the screw configuration reported in Koksel & Masatcioglu (2018). The die is a 30-cm-long cooling die custom made by APV Baker (Peterborough, UK), and uses two water circulation systems. The cross-section of the die slit is 50 mm × 5 mm. For the entire study, the feed rate (0.51 kg/h), the screw speed (200 rpm) and the temperature profile within the die and the barrel were kept constant. Based on previous literature (Lin, Huff, & Hsieh, 2002; Zahari, et al., 2020; Guo, et al., 2020; Wittek, Zeiler, Karbstein, & Emin, 2021; Jeon, Gu, & Ryu, 2023), the chosen temperature profile for the barrel from the feeder to the die was 60–80-110–130 °C, and 50–25 °C for the long cooling die. Five different feed moisture contents of 58%, 61%, 64%, 67% and 70% wet basis (wb) were studied. Torque values (%) and pressure (bar) at the entrance of the long cooling die (i.e., die pressure) were recorded four times during extrudate collection. The die pressure and torque value for each feed moisture content treatment can be found in the supplementary material (Fig. S1).

Long strips of the extrudates were collected and immediately placed in sealed Ziploc bags to prevent moisture loss and saved in a 4 °C environment for additional measurements 24 h after the extrusion run. Each feed moisture content treatment was extruded in triplicate.

2.3. Ultrasonic measurements

Ultrasonic measurements in transmission mode were performed using a SIA-7 unit (VN Instruments Ltd., Elizabethtown, ON, Canada) and two broadband capacitive transducers (CAP3 from VN Instruments Ltd.) in the 250–450 kHz frequency range. The signal sent was a chirp of central frequency 390 kHz, bandwidth 380 kHz, and duration 100 μs, so that the frequencies contained in the chirp waveform ranged from 200 kHz to 580 kHz. For other examples of chirp waveforms used in conjunction with the VN Instruments equipment, see the following references (Neeson et al., 1999; Bulman, Ganezer, Halcrow, & Neeson, 2012; Häupler, Peyronel, Neeson, Weiss, & Marangoni, 2014; Martini, Bertoli, Herrera, Neeson, & Marangoni, 2005). Note that the actual range of frequencies in the pulses emitted and detected by the transducers (central frequency 360 kHz and full-width-half-maximum bandwidth 290 kHz) is somewhat less because of the frequency response of the transducers and wave attenuation in air. Signal processing was performed within the SIA-7 unit which uses a pulse compression technique, Synthetic Impulse™, to extract the signal envelope and improve tremendously the signal-to-noise ratio as well as echo separation within the signal. The recorded data consisted of a series of consecutive signal-averaged acquisitions (with the recorded signal averaging for each acquisition being averaged over 20 pulse repetitions) on a moving meat analogue sample (during extrusion) or on static meat analogue sample (in the lab 24 h afterwards). Note that the pulse repetition frequency was sufficiently rapid that there was no measurable evolution of the signals during each acquisition when the sample was moving, allowing the signal averaging to be used to significantly improve the signal-to-noise ratio for the recorded data. For the moving meat analogue samples, two series of 40 live acquisitions were recorded during extrusion, while for the static meat analogue samples, four series of 20 static acquisitions were recorded at four different locations on an extrudate piece.

To hold the transducers face-to-face for these transmission

experiments, and to ensure the best alignment and position adjustment, two transducer holders using precision Opti-claw mounts (New Focus™, Newport, Irvine, CA, USA), and an optical rail and carriers (Edmund Optics, Barrington, NJ, USA) were used. The diameter of transducers (37.5 mm for the active surface) is close to the width of the extrudate (50 mm). To prevent the capture of signal travelling in air around the extrudate, the extrudate was supported on an aluminum inclined plane in which a slot had been machined, thereby blocking all stray signals but allowing ultrasound transmitted through the extrudate to pass through unobstructed (Fig. 1). The bottom side of the aluminum plate was layered with wrapping foam to effectively dampen any ultrasonic signals in the plate and further ensure that the only signal captured comes from propagation through the extrudate. A measurement to investigate the possible propagation of ultrasound through this screen showed that no measurable signal was transmitted, thereby confirming the screen's effectiveness. The setup (aligned transducers in their holders and inclined plane) was placed about 20 cm away from the long cooling die exit to capture the ultrasonic signature of the meat analogues. The setup was also used in the lab to perform ultrasonic measurements 24 h after extrusion, allowing the comparison of ultrasonic results with texture measurements performed at the same time and investigation of any aging related changes in the meat analogues. From the transmitted acoustic signals ultrasonic velocity was measured for each extrusion replicate both online during extrusion and in the lab 24 h after production. Prior to each series of acquisitions, a reference signal was recorded in order to measure the ultrasonic signal that travels directly from one transducer to the other with no meat analogue sample between the transducers, keeping all other conditions exactly the same.

2.4. Ultrasonic signal analysis

Ultrasonic signals can be strongly affected by the microstructure and in particular if air inclusions (bubbles or layers) are present. Over a broad frequency range around the bubble resonance frequency, their presence causes a large attenuation and rapid velocity variation of the ultrasonic wave, with the velocity being remarkably low below resonance (Koksel, Scanlon, & Page, 2016). While bubbles can be present in meat analogues in some conditions, for example when the die does not cool the material enough to prevent expansion upon die exit, the extrusion conditions used for this paper prevented the nucleation of bubbles upon die exit as the extrudate exits the die at a temperature lower than 30 °C. Therefore, the signal observed has a very low attenuation and there are multiple reflections of sound wave pulses within the meat analogue (Fig. 2).

The impedance mismatch between the surrounding air and the material is large, so most of the incoming wave is reflected back from the surface of the food (Kerhervé, et al., 2019) at the interface between the air and the meat analogue. Similarly, the small portion of the incoming wave that is transmitted through the interface into the meat analogue reverberates back and forth inside it, because when the wave reaches the opposite side of the meat analogue the wave is mostly reflected back into the sample at these sample-air interfaces. Each time the wave reaches the interface with the air outside, a fraction of the reverberating wave nonetheless escapes, giving rise to a sequence of transmitted signal pulses that are detected by the receiving transducer, as shown by the train of echoes displayed in Fig. 2. The interesting information obtained is the velocity of the ultrasonic wave and the attenuation within the meat analogue. The attenuation being very low for all meat analogues analyzed in this study (i.e., intensity attenuation coefficient α 0.02 mm^{-1}),[1] the focus is on the ultrasonic group velocity that characterizes

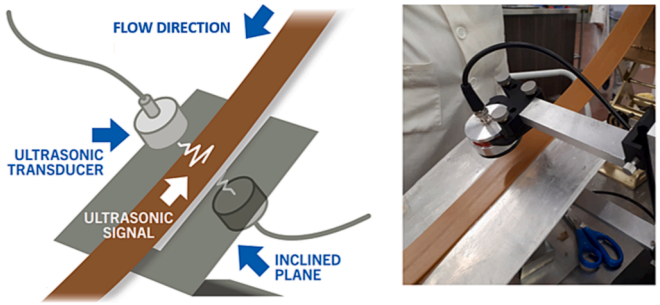

Fig. 1. Experimental setup used to study the propagation of ultrasound in high-moisture meat analogues. (Manitoba Agriculture and Food Knowledge Exchange, 2020).

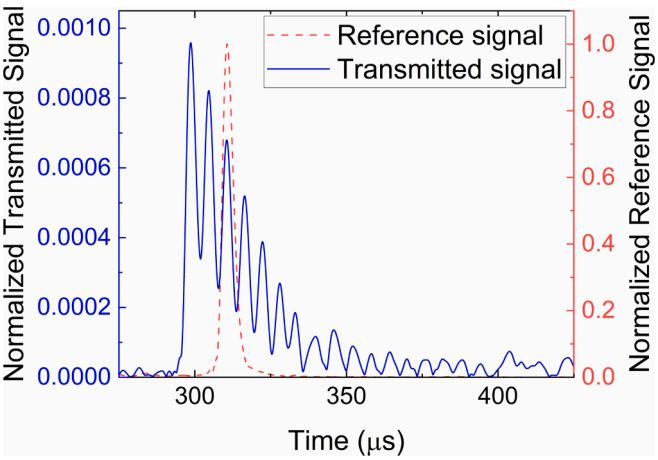

Fig. 2. Examples of a reference signal through air alone (dashed line) and a transmitted ultrasonic signal after propagation through a meat analogue sample (solid line). Both signals are normalized by the peak amplitude of the reference signal with no meat analogue sample present. The reference signal is about 1000 × larger than the signal after propagation through the meat analogue sample.

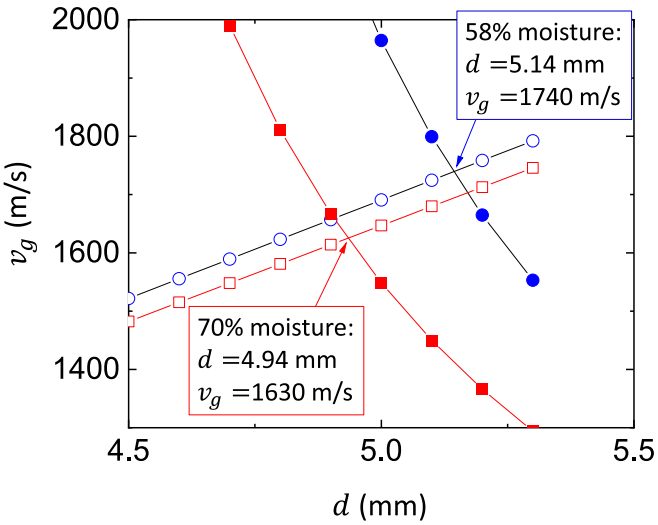

Fig. 3. Group velocity (v_g) predicted by Eq. (1) (open symbols) and Eq. (2) (closed symbols) as a function of thickness d for 58% (circles) and 70% (squares) feed-moisture-containing meat analogue samples. The intersection of the curves from both methods provides the thickness of the meat analogue sample and the group velocity. Arrows represent the correct v_g and d for 58 and 70% moisture-containing meat analogues.

[1] Recall that the intensity attenuation coefficient α is defined in terms of the intensity ratio as $I(d)/I(0) = \exp(-\alpha d)$, where $I(d)$ is the intensity after propagating a distance d, and $I(0)$ is the intensity for $d = 0$.

the speed at which a wave pulse travels through a sample (Cobus, Ross, Scanlon, & Page, 2007; Cowan, Beaty, Page, Liu, & Sheng, 1998; Koksel, Scanlon, & Page, 2016; Page et al., 1996).[2]

The group velocity v_g can be extracted from the time-dependent ultrasonic signals in two different ways. One method involves only the transmitted pulses and is done by measuring the time difference between the peaks of the multiply reflected echoes illustrated in Fig. 2:

$$v_g = \frac{2d}{\Delta t}. \quad (1)$$

Here d is the thickness of the meat analogue sample and Δt is the average time difference between two consecutive peaks, corresponding to the time interval for ultrasound to travel back and forth once through the sample (with the distance travelled being 2 d in this case). The second method takes advantage of the additional measurement of the reference signal in air without the sample in place. Then, the group velocity can also be obtained by comparing the time of flight of the signal propagating solely in air and the signal going straight through the meat analogue:

$$v_g = \frac{d}{t_{sam} - t_{ref} + d/v_{air}} \quad (2)$$

where t_{sam} and t_{ref} are the arrival times of the first-arriving peak of the transmitted signal through meat analogue sample and the peak of the reference pulse, respectively, d is the thickness of the meat analogue sample and v_{air} is the velocity of ultrasound in air, which is 346 m/s at the conditions present in this study.

In principle, the two methods must give the same value for the group velocity and either method can be used provided that precise measurements of v_{air} and d are available. While accurate data on v_{air} is possible given knowledge of ambient conditions, obtaining accurate independent information on d can be problematic. For example, the thickness measuring method using a thickness gauge is not accurate enough, because it can induce a slight compression of the sample. However, both methods of measuring v_g are extremely sensitive to d with a different dependence, as presented in Eqs. (1) and (2). By taking advantage of accurate knowledge of v_{air}, as well as the fact that the values of v_g and d depend only on the material and not which of the two analysis methods is used, accurate measurements of *both* v_g and d can be obtained by plotting the dependence of v_g on d predicted by these two expressions (Eq. (1) and Eq. (2)) using the measured values of Δt, t_{sam} and t_{ref} (Fig. 3), and extracting the values of v_g and d at the intersection of both curves. This is equivalent to solving the system of two linear equations for v_g and d, resulting in the following analytic expressions:

$$v_g = \left(1 + \frac{2(t_{ref} - t_{sam})}{\Delta t}\right) v_{air} \quad (3)$$

$$d = \left(\frac{\Delta t}{2} + t_{ref} - t_{sam}\right) v_{air} \quad (4)$$

Hence, Eqs. (3) and (4) enable direct calculation of extrudate thickness and group velocity from the arrival times of the ultrasonic pulses and the known speed of sound in air.

The significance of this technique is that ultrasonic experiments can provide an accurate measurement of both parameters at the same time and therefore a non-contact alternative way of accessing the thickness of the meat analogue produced, as well as the group velocity.

[2] More technically, the group velocity is defined as the derivative of the angular frequency ω of a wave with respect to its wavevector k, i.e., $v_g = \partial\omega/\partial k$. Experimentally, under conditions that apply to these measurements, it is measured from the ratio d/t of distance d travelled by a wave pulse to the time t taken for the peak of the wave pulse to travel this distance. See the experiment section of Cowan et al., 1998, and Koksel, et al., 2016 for additional details.

2.5. Texture analysis

Textural properties were measured, 24 h after extrusion, using a texture analyzer (TA-XT-plus, Stable Micro Systems, Godalming, UK) with an A/ECB blade probe, following the method of Osen, Toelstede, Wild, & Schweiggert-Weisz (2014) with modifications. A cutting test with the following settings was performed: pre-test speed: 1 mm/s, test speed: 2 mm/s, post-test speed: 10 mm/s, cutting distance: 75% of sample thickness. For each meat analogue sample, sixteen 20 × 20 mm pieces were extracted from the centre of the extrudate. These pieces were alternatively tested in the longitudinal direction (along the extrusion flow) and in the transverse direction (across the extrusion flow), so in total there were 8 × 3 = 24 measurements for the longitudinal cutting force and 24 measurements for the transverse cutting force per treatment. The maximum cutting force was obtained from the force versus distance graph.

2.6. Density and thickness measurements

Density was measured in triplicate with a water displacement method using a 25 mL specific gravity bottle (Kimble Glass Inc., Vineland, NJ, USA). Thickness measurements were also performed in triplicate using a thickness gauge (Model: 547-500S, Mitutoyo, Japan) 24 h after extrusion.

2.7. Nutritional quality

Meat analogues samples were freeze-dried, ground to pass through a 1 mm screen, and stored in airtight containers at −20 °C prior to analysis. The percent nitrogen (AOAC Official Method 968.06) of the dried meat analogue samples was measured according to established procedures (Association of Official Analytical Chemists, 1995) using a nitrogen conversion factor of 6.25. With the exception of the sulphur amino acids (AA) cysteine and methionine, and tryptophan, samples were prepared as per AOAC Official Method 982.30, with 6 N hydrochloric acid hydrolysis over 24 h. Cysteine and methionine were analyzed according to AOAC Official Method 985.28, using a performic acid pre-oxidation step prior to acid hydrolysis. The resulting AAs were derivatized and separated (AccQ-Tag Ultra C18, 1.7 μm column) using the AccQ-Tag Ultra system (Waters Ltd., Mississauga, ON, Canada) chemistry (Astephen, 2018) on a Shimadzu UPLC system, complete with an SIL-30AC autosampler. For tryptophan, samples were analyzed using ISO protocol 13904, which included an alkaline hydrolysis step (International Organization for Standardization., 2016). For quality control, the NIST soy flour Standard Reference Material 3234 was used for all AA analyses. The hydrated molecular weights of AA were used to express their content in mg quantities.

The *in vitro* protein digestibility (IVPD, %) of the meat analogue samples was determined according to methods established previously (Nosworthy, et al., 2017), using a revised regression equation (Franczyk, 2018). In brief, the method involves a static digestion protocol and measures the change in pH as a function of protein digestion (Tinus, Damour, Van Riel, & Sopade, 2012).

The amino acid score (AAS) was determined by comparing the AA composition (mg/g protein) of each meat analogue sample to the recommended FAO/WHO reference pattern (AA requirements of 2–5 year old children; (FAO/WHO, 1991) (mg/g protein: Histidine = 19; Isoleucine = 28; Leucine = 66; Lysine = 58; Threonine = 34; Tryptophan = 11; Valine = 35; Phenylalanine plus tyrosine = 63; Methionine plus cysteine = 25). The lowest calculated AA ratio (limiting AA) was considered as the AAS. The final *in vitro* PDCAAS (IVPDCAAS) value was calculated as the product of the AAS and the *in vitro* protein digestibility.

2.8. Statistical analysis

Data for indices of protein quality were analyzed by one-way ANOVA

using GraphPad Prism 8.0 (GraphPad Software LLC). If the overall F-test was significant ($p < 0.05$), individual means were subjected to a multiple comparison procedure using Tukey's Honest Significant Difference method. Pearson correlation analysis was performed for the ultrasonic, textural and protein quality values.

3. Results and discussion

3.1. Effects of feed moisture content on the ultrasonic velocity and meat analogue thickness

The effect of feed moisture content (mc) on the group velocity (v_g) is presented in Fig. 4 for acquisitions performed online during extrusion and statically 24 h after extrusion. These measurements of the ultrasonic velocity show that it decreased with moisture content, due to a combination of how moisture content affects the mechanical properties and the density of the meat analogue samples. More specifically, the magnitude of the ultrasonic velocity is governed at the microscopic level by the molecular masses and the local stiffness, which in turn depends on the strength of the intermolecular interactions and their separation. Hence the velocity depends on density and elasticity and is therefore a good probe of material properties (Wood, 1955). Given that one of the widely accepted mechanisms (Wittek, Zeiler, Karbstein, & Emin, 2021) responsible for development of anisotropic structures during high-moisture meat analogue production involves formation of a continuous protein-rich (i.e., water-poor) phase and a water-rich phase dispersed in this continuous phase in the long cooling die, it is not surprising that meat analogue elasticity would be influenced by the level of water, i.e., feed moisture content, in this multiphase morphology. In addition, since the density of water is relatively lower compared to the densities of isolated grain proteins and starches (for example, approximately 1285 and 1470 kg/m^3 for vital wheat gluten and wheat starch, respectively (Koksel & Scanlon, 2012), changes in meat analogue density is expected as a function of the feed moisture content. The group velocity results were similar between the two acquisition conditions (online vs. 24 h after extrusion), indicating that meat analogue samples did not substantially change in 24 h.

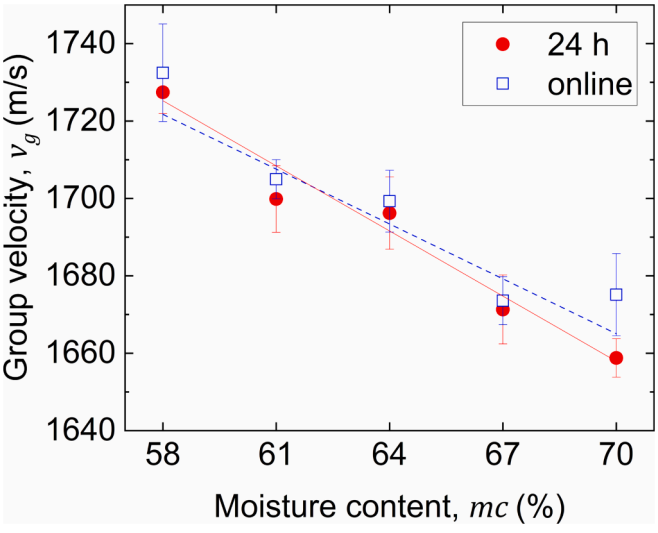

Fig. 4. Group velocity (v_g) for acquisitions performed on the meat analogues online during extrusion (open squares) and statically 24 h after extrusion (closed circles) as a function of feed moisture content. The error bars correspond to the standard error of the mean ($n = 6$ for online and $n = 12$ for 24 h afterwards). The lines are linear fits for the group velocity measured online (dashed) and 24 h after extrusion (solid). Equations are respectively $v_g = 2051 - 5.6mc$ ($R^2 = 0.98$) and $v_g = 1996 - 4.7mc$ ($R^2 = 0.85$), where mc is the moisture content.

The effect of moisture content on the meat analogue thickness measured ultrasonically online during extrusion and statically 24 h after extrusion, as well as measured using the thickness gauge 24 h after extrusion is presented in Fig. 5. For the meat analogue thickness measured online ultrasonically, the thickness was relatively constant across the moisture range studied. For the thickness measurements performed on the 24-h-old meat analogue samples, both ultrasonic and thickness gauge methods indicate a decrease in thickness with increasing feed moisture content and a good agreement between the two methods, especially at relatively lower feed moisture contents. However, for higher moisture-containing meat analogue samples, which are also softer, a slight divergence appears between the thickness of meat analogues obtained from thickness gauge measurements and ultrasonic measurements performed 24 h after extrusion, which is very likely due to the compression induced during the thickness gauge measurements. In any case, the thickness variation over the entire range of moistures is small (4.95 mm – 5.15 mm) and it can hardly be resolved up by simple visual observation.

The high quality of the ultrasonic data obtained both during extrusion and later in a lab configuration (24 h after extrusion) shows that the technique is reliable and applicable in real industrial settings. Sound velocity in air is influenced by temperature and atmospheric pressure, so care needs to be taken to measure these parameters and to apply the appropriate corrections during the analysis step, and ideally to have well controlled atmospheric conditions. The fact that the thickness can be measured ultrasonically without contact is extremely interesting for quality control purposes to check the stability of meat analogues in a sanitary manner during production and also to detect production-related issues in various sheeted and extruded foods (e.g., uncontrolled folds on noodle production lines, unwanted air pockets in pasta production lines).

3.2. Effects of feed moisture content on the textural quality attributes of meat analogues

The effect of moisture content on the longitudinal and transverse peak cutting forces of meat analogues is presented in Fig. 6. Overall, the transverse cutting force was equal to or larger than the longitudinal cutting force. According to Osen, Toelstede, Wild, & Schweiggert-Weisz

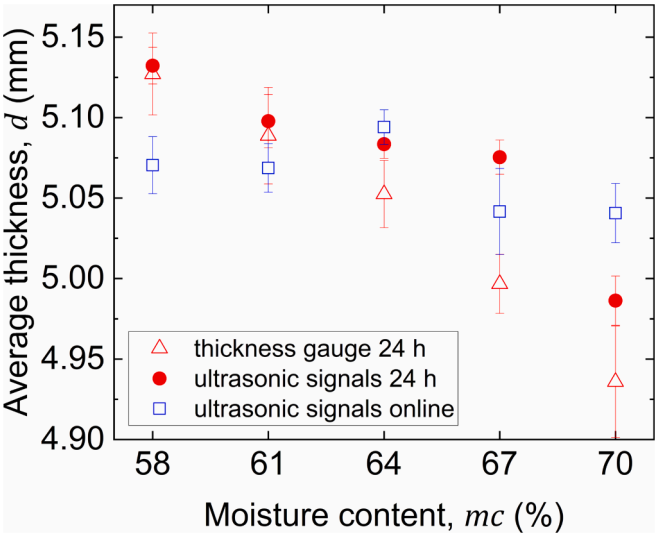

Fig. 5. Average meat analogue thickness measured with the ultrasonic method online during (closed squares) and statically 24 h after extrusion (closed circles) and with the thickness gauge (open circles). Error bars represent the standard error of the mean ($n = 6$ for ultrasonic thickness measurements online, $n = 12$ for ultrasonic thickness measurements after 24 h and for thickness gauge measurements).

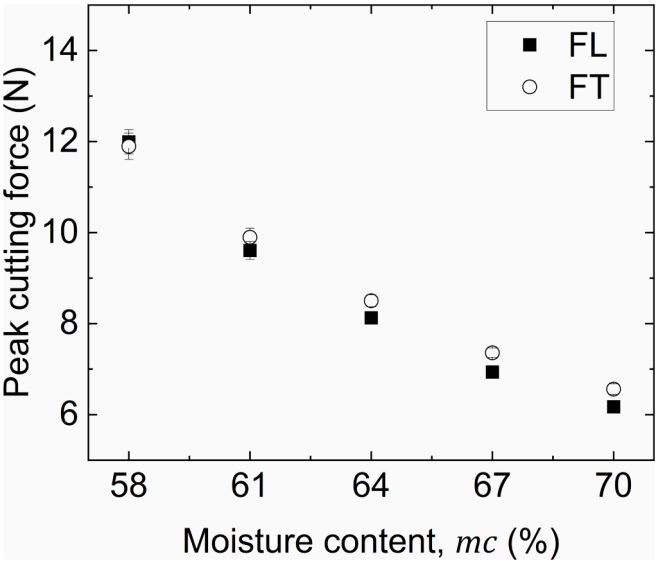

Fig. 6. Longitudinal (FL) and transverse (FT) peak cutting force of meat analogues as a function of feed moisture content. Error bars represent the standard error of the mean ($n = 24$).

(2014), the difference between the two cutting forces is an indication of texturization for pea-based meat analogues, i.e., the higher the difference the better the texturization. Given that the peak cutting force values obtained in either direction are very similar, with a small difference between the two peak cutting forces at larger moisture content, one might expect little to no texturization in the meat analogues in this study. However, visually, the meat analogues presented an obvious texturization for all extrusion conditions studied, which means that the texturization is heterogeneous. This reveals a difference in the texturization behavior of pea proteins when texturized alone and the soy-gluten protein blend studied here, in line with the literature on soy-protein-containing plant-based meat analogues (Schreuders, et al., 2019; Wang, et al., 2022). As seen in Fig. 6, the peak cutting force is decreasing with moisture. This is in agreement with other studies on the texture of meat analogues made from soy protein (Lin, Huff, & Hsieh, 2002), soy and hemp protein mixtures (Zahari, et al., 2020), yellow pea and faba bean protein mixtures (Ferawati, et al., 2021), faba bean protein (Kantanen, et al., 2022), and lupin protein (Palanisamy, Franke, Berger, Heinz, & Töpfl, 2019).

For figure clarity, only the longitudinal cutting force will be subsequently presented. To explore the link between the velocity and the peak cutting force, in Fig. 7, group velocity, v_g, was plotted against longitudinal peak cutting force (FL). The data are clearly correlated, and a linear fit can connect these two variables with a very high coefficient of determination ($R^2 = 0.95$).

The fact that the two variables, v_g and FL, are correlated can be explained by the link between ultrasonic velocity and mechanical moduli. Strictly speaking, it is the phase velocity of the wave oscillations (v_{ph}) rather than the group velocity of the wave pulse (v_g) that is directly related to the elastic modulus (Koksel, Scanlon, & Page, 2016), but for these low-attenuating, non-resonant meat analogue samples, we find from additional analysis to be presented in a subsequent publication that these two velocities are equal within error bars. Hence our measurements of $v_g = v_{ph} \equiv v$ can be used to calculate the modulus, and this velocity can be written as:

$$v = \sqrt{\frac{M}{\rho}} \tag{5}$$

where M is the longitudinal modulus and ρ is the density of the meat analogue. The longitudinal modulus can be written as a function of the

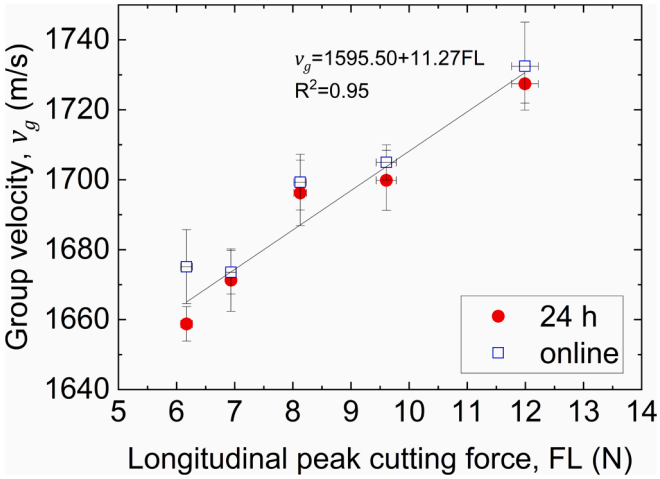

Fig. 7. Group velocity (v_g) as a function of longitudinal peak cutting force (FL). The line is a linear fit on concatenated data from online (open squares) and 24 h (closed circles) ultrasonic measurements. Error bars represent the standard error of the mean ($n = 24$ for FL and $n = 12$ for v_g).

bulk modulus K and the shear modulus G through $M = K + \frac{4G}{3}$.The action of cutting a sample with a blade also involves compression and some shear, with the complete physical description of the process being complex (Boisly, Schuldt, Kästner, Schneider, & Rohm, 2016) and the measured force inevitably depending on instrument-specific details such as the type and shape of the probe used (e.g., cutting wire vs. knife blade). Nonetheless, since both ultrasonic modulus and peak cutting force depend on the mechanical response of the medium to applied forces (stress, or force per unit area, in the case of elastic modulus), it is logical scientifically that there be a relationship between the cutting force and the ultrasonic velocity. Furthermore, since both cutting force and velocity measure how a material is deformed when a force is acting on them, and hence probe in different ways the strength of the cohesive intermolecular interactions holding a material together, both methods must provide a means of assessing product firmness. One advantage of the ultrasonic approach is that it measures a fundamental property of the material (elastic modulus) in SI units irrespective of the particular ultrasonic technique or instrument used. However, some attention should be given to the different time scales involved: the cutting action time scale is on the order of seconds, while the ultrasonic time scale is on the order of 10 μs (the corresponding frequency is about 100 kHz). Despite these different scales, the linear interpolation of the velocity versus cutting force is very good and this relationship provides a good route towards the development of an online tool to assess the firmness/hardness of high-moisture meat analogues.

Equation (5) can also be used, provided that information about the density of a material can be measured independently, to obtain the longitudinal modulus for the ultrasonic range of frequencies involved. This equation implies a negligible attenuation which is the case in this particular study, for which some trials indicated an attenuation coefficient of only 0.02 mm^{-1} for a typical meat analogue sample. If the attenuation were not negligible, a complex modulus would need to be considered (Kerhervé, et al., 2019), but this is not necessary here, since the attenuation for these meat analogue samples is so small that M defined by Eq. (5) differs from the real part of the complex longitudinal modulus by <0.01% (with the longitudinal loss modulus being less than ~ 1% of the longitudinal storage modulus). Fig. 8 shows the evolution of the ultrasonic longitudinal modulus as a function of the moisture content of the meat analogue samples. A linear fit is also shown and captures the data points well with a high coefficient of determination ($R^2 = 0.999$). The small differences between the ultrasonic longitudinal modulus measured online and offline 24 h after extrusion indicate that the mechanical properties of the meat analogues did not change

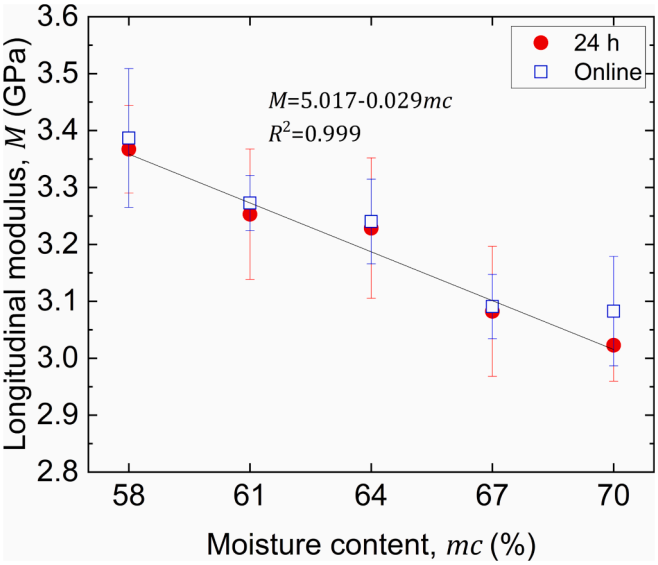

Fig. 8. Longitudinal modulus M as a function of moisture content. Error bars represent the standard deviation (from propagation of error calculations). Black line with the equation $M = 5.017 - 0.023mc$ is a concatenated fit using online (open squares) and 24 h (closed circles) ultrasonic measurements and mc is the moisture content of the meat analogues.

significantly due to aging.

3.3. Effects of feed moisture content on the nutritional quality of meat analogues

In all work on foods, it is important to verify that processing conditions do not damage nutritional quality. As depicted in Table 1, there was a significant ($p < 0.05$) effect of the feed moisture content on the content of several indispensable amino acids, including the branched chain amino acids (isoleucine, leucine, and valine), lysine, aromatic amino acids (phenylalanine + tyrosine) and threonine, of the meat analogues. Where significant, increasing the feed moisture content led to increased levels of the respective amino acids, expressed on a mg/g protein basis, with the highest values observed at 70% feed moisture. The fact that the intensity of extrusion cooking, as influenced by moisture content, alters extrudate nutritional quality is very widely documented in the literature (Singh, Gamlath, & Wakeling, 2007; Qi & Onwulata, 2011; Zhang, et al., 2018). Changes such as the inactivation of anti-nutritional factors and improvement of protein and starch digestibility are brought about by moisture content's impact on the die pressure and torque values, and how they interact influence food polymers (e.g., starch gelatinization, protein denaturation) during extrusion. Die pressure and torque values as a function of feed moisture content were provided in the supplementary material (Figure S1) and showed approximately 25 bar drop in die pressure and 12% drop in torque when moisture content is increased from 58 to 70%. In line with our findings, previous studies have also observed that thermally processing pulses at a higher moisture content (boiling, extrusion) was more protective of the amino acid content than dry heating methods (baking) (Nosworthy, et al., 2017).

Expressing the amino acid content relative to the FAO/WHO reference pattern for 2–5 year school children (FAO/WHO , 1991), the required regulatory pattern for protein content claim substantiation in the US and Canada, revealed that lysine was the limiting amino acid across all treatments, with the lowest AAS observed at the lowest feed moisture content. The resultant AAS values indicated that the balance of the amino acids in the original extrusion feed (soy protein and wheat gluten) mixture was complementary. It is also noteworthy that to carry any protein content claim substantiations, foods need to have a certain amount of in vivo PDCAAS-corrected protein per RACC, where RACC refers to the reference amounts customarily consumed (Food and Drug Administration, 2013). Since RACC values for specific food types are predetermined (for example, 55 g for meatless substitutes for sausages, seafood, luncheon meat, etc.), a relatively lower moisture content per the reference amount would mean a relatively higher protein concentration, and vice versa. Accordingly, the moisture content of high-moisture meat analogues would directly affect possible nutritional claims.

To fully assess the protein quality of the food protein source, a measure of digestibility is required. In the absence of true fecal protein digestibility measures, the required measure for official PDCAAS assessments, the current study employed an *in vitro* assessment protocol. This method has been shown to be sufficiently sensitive to detect changes in protein digestibility with good agreement to in vivo estimates (Nosworthy, et al., 2017; Nosworthy, et al., 2020). Feed moisture content did not significantly impact *in vitro* protein digestibility (IVPD) values, in line with an investigation on meat analogues made from another protein source (Palanisamy, Franke, Berger, Heinz, & Töpfl, 2019). As such, the resultant *in vitro* PDCAAS, the product of the AAS and IVPD, mirrored the results for the AAS, with higher values observed in the highest feed moisture treatment (Table 1). Overall, the resultant high-moisture meat analogues possessed final *in vitro* PDCAAS values between 0.88 and 0.93, indicative of a food protein with high indices of protein quality.

Correlation analysis of the protein quality assessments with the ultrasonic measurements (online and 24 h after) and the textural quality

Table 1
Impact of high-moisture meat analogue feed moisture content on indices of protein quality.

	Moisture content					
	58%	**61%**	**64%**	**67%**	**70%**	**p value**
Histidine	23.00 ± 0.28	23.93 ± 0.63	24.27 ± 0.53	22.06 ± 0.53	22.96 ± 0.46	>0.05
Isoleucine	43.12 ± 0.35^{a}	43.26 ± 0.12^{a}	43.42 ± 0.28^{a}	43.17 ± 0.16^{a}	44.66 ± 0.24^{b}	0.0063
Leucine	73.98 ± 0.46^{a}	73.89 ± 0.13^{a}	75.41 ± 0.41^{a}	74.22 ± 0.22^{a}	77.24 ± 0.35^{b}	0.0002
Lysine	55.68 ± 0.24^{ab}	55.16 ± 0.52^{a}	57.16 ± 0.50^{bc}	56.34 ± 0.46^{ab}	58.29 ± 0.17^{c}	0.0021
Methionine + Cysteine	26.83 ± 0.26	27.40 ± 0.47	27.13 ± 0.41	26.50 ± 0.53	27.91 ± 0.34	>0.05
Phenylalanine + Tyrosine	82.17 ± 0.84^{a}	83.46 ± 0.58^{ab}	82.74 ± 0.94^{ab}	81.95 ± 0.29^{a}	85.62 ± 0.76^{b}	0.0297
Threonine	34.36 ± 0.15^{a}	34.17 ± 0.05^{a}	35.22 ± 0.51^{ab}	34.30 ± 0.09^{a}	35.72 ± 0.09^{b}	0.0045
Tryptophan	10.87 ± 0.20	11.27 ± 0.25	11.33 ± 0.32	11.17 ± 0.08	11.30 ± 0.24	>0.05
Valine	44.27 ± 0.21^{ab}	44.20 ± 0.06^{a}	45.01 ± 0.27^{b}	44.31 ± 0.09^{ab}	46.00 ± 0.09^{c}	<0.0001
Amino Acid Score	0.96 ± 0.00^{a}	0.95 ± 0.01^{a}	0.98 ± 0.01^{ab}	0.97 ± 0.01^{ab}	1.00 ± 0.01^{b}	0.0049
IVPD	92.40 ± 0.20	93.00 ± 0.05	92.61 ± 0.38	93.18 ± 0.33	93.21 ± 0.27	>0.05
IVPDCAAS	0.88 ± 0.01^{a}	0.88 ± 0.01^{a}	0.90 ± 0.01^{ab}	0.91 ± 0.01^{ab}	0.93± 0.01^{b}	0.0116

Values (mg/g protein) are presented as means ± standard error (n = 3). Values within a row with different superscripts are significantly different ($p < 0.05$). IVPD = *in vitro* protein digestibility; IVPDCAAS = *in vitro* protein digestibility corrected amino score.

attributes provided evidence of significant Pearson correlations (supplementary material, Table S1). The strongest correlation (r = -0.646) was observed for the relationship between *in vitro* PDCAAS values and online longitudinal modulus, *M*. The inverse relationship between the online ultrasonic velocity, v_g, for example, and the measures of protein quality are worthy of further evaluation, particularly with different feed mixtures, as a means to predict real-time processing effects on protein quality. However, at this stage, it would be speculative to infer a causal relationship as these same measures are also highly correlated to the feed moisture content.

4. Conclusions

This study shows the feasibility of using a non-contact low-intensity ultrasonic technique to monitor high-moisture meat analogue quality during production. In particular, this technique is able to detect changes in moisture content of meat analogues and to precisely measure their thickness. It is worth emphasizing that the non-contact ultrasonic method introduced here for measuring the thickness of low-attenuation samples without touching the sample is potentially very valuable, since, for soft materials, normal contact methods using a thickness gauge can produce erroneous results due to sample compression during the measurement. A very strong correlation between the peak cutting force obtained from texture measurements and the ultrasonic velocity was demonstrated. Furthermore, a relevant mechanical parameter, namely the longitudinal storage modulus, can be measured directly using the ultrasonic velocity and independently measured density of the meat analogues. The relationship between velocity and modulus provides a link to understanding why velocity and peak cutting force are strongly correlated, and identifies the mechanism underpinning the use of ultrasonic velocity to assess product firmness. Correlation analysis showed inverse relationships between the indices of protein quality and ultrasonic velocity. Future studies with different feed mixtures and moisture contents will strengthen the understanding as to whether ultrasonic measurements can be predictive of changes in protein quality in real time during production.

For online ultrasonic experiments, care needs to be taken to closely monitor the atmospheric conditions (temperature and pressure) as the analysis is sensitive to the velocity of ultrasound in air. Depending on the extrusion conditions, the analysis method may need to be adapted, and the central frequency of ultrasonic waves might need to be adjusted. Especially, if bubbles are present, the attenuation could become large enough to prevent any multiple reflections within the meat analogue sample, and the signal-to-noise ratio might become too small for adequate signal detection without modifying the experimental setup. In these conditions, lowering the frequency of the ultrasonic waves or reducing the vertical dimension of the die (which translates to meat analogue thickness) are strategies for overcoming such challenges. The technique reported here has been tested multiple times in various conditions and all data are consistent with what is presented in this paper.

Our results point to the potential of this technique to advance the real-time quality control of processing plant-based meat alternatives through automation support of already existing manufacturing facilities. The same tool can then be later modified to fit different equipment geometries for a wide variety of products. Future work will focus on the use of this technique on a variety of meat analogues made from pea protein or other protein sources, and an upscale towards industrial applications.

CRediT authorship contribution statement

R.-M. Guillermic: Investigation, Conceptualization, Methodology, Formal analysis, Software, Writing – original draft, Writing – review & editing, Visualization. **A.J. Franczyk:** Investigation, Formal analysis, Writing – original draft. **S.O. Kerhervé:** Conceptualization, Software. **J. D. House:** Resources, Writing – original draft, Supervision. **J.H. Page:** Conceptualization, Methodology, Writing – review & editing. **F. Koksel:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113193.

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