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New contributions of ultrasound inspection to the characterization of different varieties of honey

Alberto González-Mohino[a,*], Antonio Jiménez[b], M. Jesús Paniagua[b], Trinidad Perez-Palacios[a], Montaña Rufo[b]

[a] *Department of Food Technology, Research Institute of Meat and Meat Product, University of Extremadura, Avenida de la Universidad s/n, 10003 Cáceres, Spain*
[b] *Department of Applied Physics, School of Technology, University of Extremadura, Avenida de la Universidad s/n, 10003 Cáceres, Spain*

1. Introduction

Honey is a natural sweet product produced by the bee *Apis mellifera* from secretions of plants. The composition is mainly several types of highly assimilable monosaccharides (with the greatest proportions being of fructose and glucose) [1]. It is noted for its nutritional value and therapeutic properties [2]. Currently the different varieties of honey that are usually found on the market are multifloral or monofloral, the latter being those that are more appreciated by the consumer [3].

Honey has suffered numerous cases of adulterations and fraud throughout history because it is a product of limited production. Therefore, characterizing it and determining its quality, as well as being able to distinguish between multifloral and monofloral varieties, is of utmost importance to protect both the producer and the consumer. There are many analytical methods used to check the authenticity of honeys [4]. However, most are destructive techniques. Moreover, some, such as microscopic analysis, are not valid for all types of honey varieties, and require an expert for their application [5].

Ultrasound inspection has been widely used to analyse different foodstuffs due to its non-destructive character. Examples are found in several studies in the literature, for example, to determine the composition of a cod sample [6], to detect microbial contamination in juices [7], and to monitor the milk curdling process [8]. The ultrasound pulse velocity (UPV) has also been used to determine the physical and mechanical properties of honey. Singh et al. [9] were able to demonstrate adulterations in honey from the speed of ultrasound passing through the samples. More recent studies have used this parameter to differentiate honeys, making the measurements at different temperatures [10,11]. Additionally, moisture has been determined in honey samples by applying high-frequency dynamic shear rheology [12]. There are other parameters that can be extracted from ultrasound, such as attenuation, but they have not been subject to assessment in the scientific literature.

Texture related parameters have a great impact on food quality. Texture profile analysis (TPA) is a technique widely used to assess instrumental texture characteristics, and it has been applied to several food products [13,14] including honey [15].

The objective of the present study was to use ultrasound inspection to characterize and differentiate between different types of honey (Heather, Thyme, Eucalyptus, and Thousand Flower), all from Extremadura (Spain), at different temperatures (25 °C, 30 °C, 35 °C, 40 °C, and 45 °C). The ultrasound parameters assessed were: longitudinal ultrasound pulse velocity (UPV_L) and attenuation. To the best of our knowledge, attenuation has not previously been considered in other work. Additionally, a textural study was carried out, and its results were correlated with the ultrasound parameters (by means of a linear statistical correlation analysis). As our knowledge, there is not studies that correlate data from both techniques in the scientific literature.

This research is intended to contribute to knowledge about the acoustic properties of honey, providing a new parameter for study that had not been considered previously for this product (attenuation). The information obtained we believe should be invaluable for distinguishing different types of honey. This opens up a new path not just to characterizing different varieties of honey with ultrasound but to using it as a non-destructive, fast, and effective method with which to ensure the authenticity of the honey and thus avoid possible fraud in the market.

2. Materials

The honey samples, purchased in a Spanish supermarket, belonged to the “Sabores del Guijo” brand (produced in Cáceres, Extremadura). They correspond to four varieties: monofloral Heather, Thyme, and Eucalyptus, and multifloral Thousand Flower. The samples studied were of 100 ml evaluated in a beaker of 120 ml capacity. The honeys were heated to 45 °C in a thermostatic bath (JP SELECTA, Barcelona, Spain). Beginning from this 45 °C temperature, ultrasound inspections were carried out every 5 °C as the sample gradually cooled until 25 °C. The inspections were done in duplicate with different jars. Thus, a total of ten measurements were thus made for each sample.

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[*] Corresponding author at: IPROCAR, Avd Ciencia s.n, 1003 Cáceres, Spain.
E-mail address: albertogj@unex.es (A. González-Mohino).

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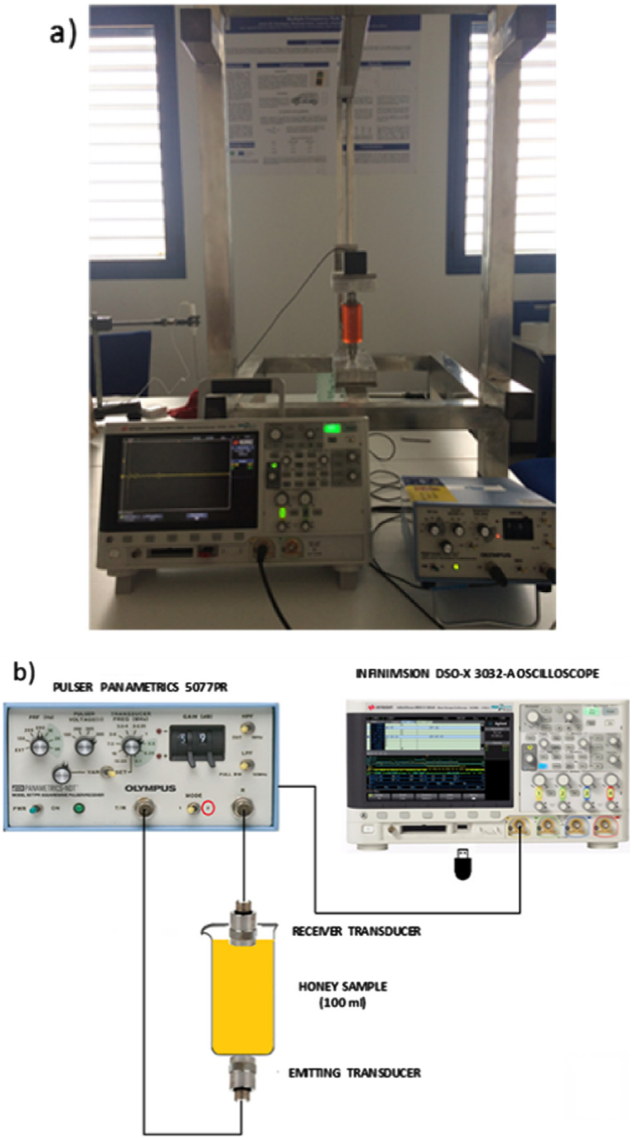

Fig. 1. Experimental set-up used for the measurements of the ultrasound parameters, picture (a), and diagram (b).

3. Methods

With respect to the ultrasound parameters, the samples were inspected using immersion techniques in through-transmission (TT) mode. Fig. 1 shows the set-up used for these measurements. The immersion transducers were single-element longitudinal wave transducers, with wear surfaces impedance matched to honey. They were mounted on a custom-designed metal structure that ensured their perfect face-to-face alignment, also reducing the risk of other errors being introduced by the operator. One of the transducers acted as a mirror reflecting the ultrasound waves. Whether this inspection mode or that of pulse-echo is used is irrelevant since they give similar results [8]. In particular, the transducer that acts as a transmitter was placed at the base of the beaker, whence it transmits the wave through the coupling gel and the glass of the base. The wave travels through the honey and reaches the transducer that acts as a receiver located at the upper part of the beaker, immersed approximately 1.5 cm in the honey. The ultrasound signals were transmitted and received using an Olympus Pulser-Receiver Panametrics-NDT Model 5077PR. The pulser section can deliver up to 400 V, and the range of pulse repetition rates is from 20 Hz to 5 kHz. The pulse rise time is typically less than 10 ns. The flexible receiver section provides a wide dynamic range from 1 to 59 dB gain, with 1 dB sensitivity adjustments. The signal response is optimized by two-position high- and low-pass filters (out or 1 MHz, and out or 10 MHz, respectively), and 1 dB attenuation ranges from 0 to 49 dB. For the acquisition and digitalization of the signals, the pulser-receiver was connected to an InfiniiVision DSO-X 3032A oscilloscope from KEYSIGHT (350 MHz bandwidth, 4 GSa/s sample rate), 10,000 points record length which stored the data (.csv format) of the displayed signals for subsequent processing and analysis. Olympus Panametrics-NDT Model V318-SU piezoelectric transducers were used to transmit the signals. Table 1 lists the main characteristics of these transducers, including the near-field zone and the beam-spread angle. The near-field is the region directly in front of the transducer where the sound field pressure varies widely. Because of these variations, it can be difficult to accurately assess flaws when using amplitude-based techniques in this zone. In the present study, the separation between transducers was 85.00 mm, which ensured the consistency of the measurements. The beam-spread angle refers to the divergence of the sound beam as it travels through a medium (the solid angle which contains the main lobe of the beam in the far field). Once all the necessary instruments and devices were in place, the various ultrasound parameters were determined.

3.1. Ultrasound parameters

As noted above, the ultrasound parameters determined were the longitudinal ultrasound pulse velocity UPV_L (or time-of-flight, TOF), and attenuation.

Fig. 2 shows a typical A-scan in which *i* consecutive echoes were caused by reflections between the transducers facing each other. Among other things, this emitted-received signal acquired by the oscilloscope allowed the UPV_L within the honey samples to be determined. The calculation is based on the Fourier transform. This transform gives a frequency domain representation of the amplitude and phase of a continuous signal acquired in the time domain. Direct calculation of the discrete Fourier transform would be computationally very time intensive, but there are various algorithms that speed up the process. Among them is the FFT presented by Cooley & Tukey in 1965 [16]. Today, there are signal processing devices which rapidly calculate the FFT, as was the case in the present study. The procedure used to calculate and plot the FFT identifies the frequency range covering the received ultrasound pulse signal and the central frequency (that of maximum amplitude). By way of example, Fig. 3 shows the FFT obtained from the A-scan of Fig. 2. This generated spectrum is used to derive additional properties of the object under investigation. The influence of a periodic excitation (here multiple echoes) shows up as maxima in the spectrum at multiples of the fundamental frequency, and

Table 1
Characteristics of the transducer model used.

Model	Diameter (cm)	Frequency (kHz)	−6 dB bandwidth (%)	N (cm)	φ_L (°)
Panametrics V318-SU	1.9	500	61.93	2.12	15.84

The value UPV_L = 2125 m/s was taken for the calculation of N and φ.
N = near-field length.
φ = beam angle.

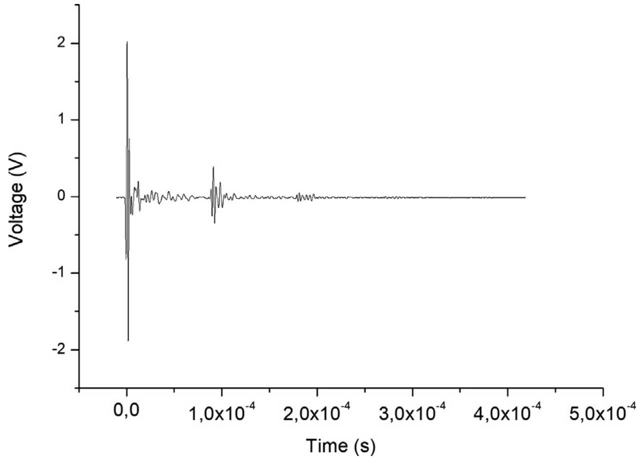

Fig. 2. Example of a typical A-scan received by the Panametrics V318-SU transducer for Thyme honey. One observes the echoes of the ultrasound signal after the trigger pulse.

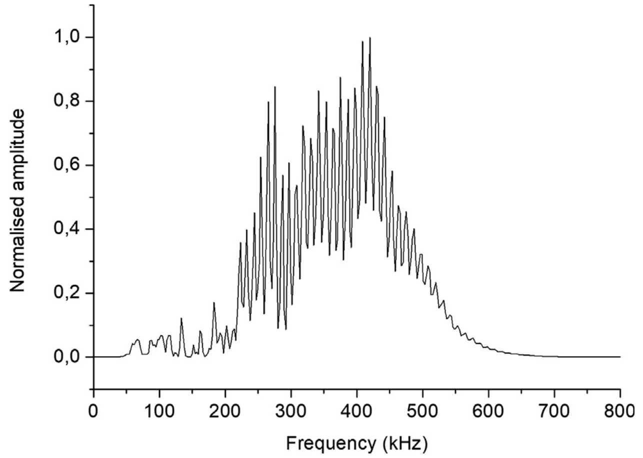

Fig. 3. FFT of the signals received in the inspection shown in Fig. 2.

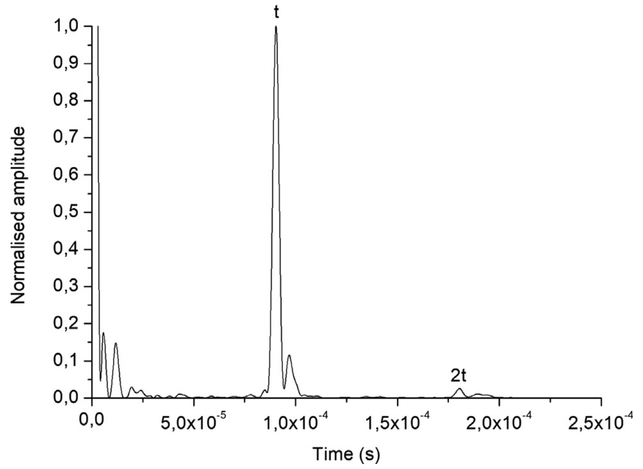

Fig. 4. Cepstrum corresponding to the FFT shown in Fig. 3. The t and $2t$ moments corresponding to the periodic excitations of the FFT are shown.

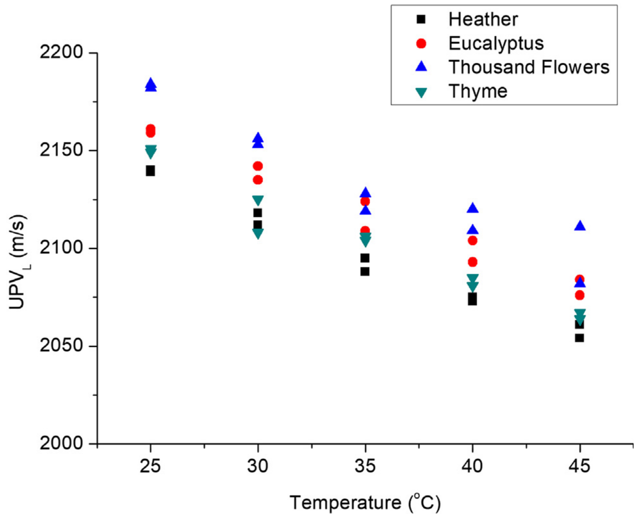

Fig. 5. Evolution of UPV_L with temperature for both batches.

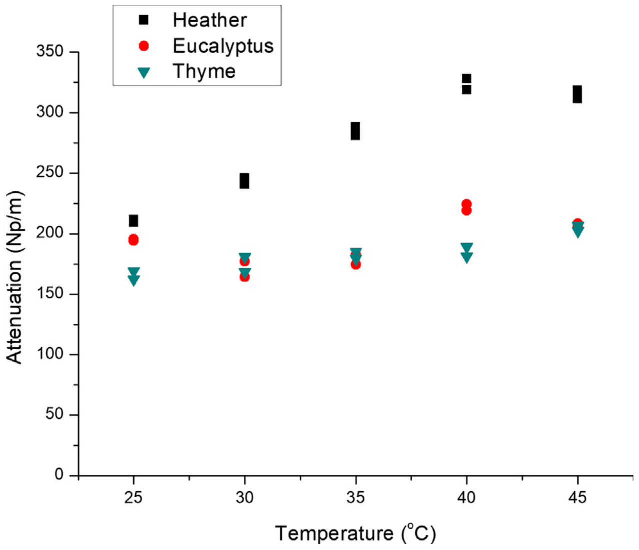

Fig. 6. Evolution of attenuation with temperature for both batches.

thus as superpositions of the fundamental spectrum of the probe with an undulation in the form of equidistant maxima in the spectrum. The TOF between the reflections is obtained from the distances of the maxima. By means of the so-called cepstrum method, the spectrum can be smoothed and the length of the period determined directly. The cepstrum comes from the inverse Fourier transform of the logarithm of the spectrum [17]. As can be seen in the example of Fig. 4, which shows the cepstrum created from the FFT of Fig. 3, the period t is read directly from the first maximum. Since the transducers are separated by a distance $d = 85.00$ mm, spectral evaluation methods provide a measurement of the UPV_L. In particular,

$$UPV_L = \frac{2d}{t} = \frac{d}{\mathrm{TOF}}$$

Obviously, for times-of-flight equivalent to $2t$, $3t$,…, nt, other maxima must also appear in the cepstrum. These serve to confirm the appropriateness of the value assigned to t. By way of example, Fig. 4 shows only the maxima corresponding to t and $2t$ because the time interval originally selected in the acquisition of the A-scan does not permit the range in the time domain to be extended to show more maxima.

Recent studies [18] support the suitability of using this method to calculate the speed of the sound waves as against the traditional computation from the times obtained for consecutive echoes in the receiver's A-scan, or even more simply, from the arrival time of the first echo [19]. This is because the errors involved in a cepstrum analysis are smaller. In the present study, the errors in the cepstrum determination of UPV_L were less than 0.096%.

Table 2
Texture parameters of honey as affected by variety and temperature of analysis (mean values ± SD).

Honey variety	Temperature	Textural parameters			
		Hardness (N)	Adhesiveness (N × s)	Gumminess (N)	Chewiness (N)
Heather 1	25 °C	4.09 ± 0.65	−7.02 ± 1.25	3.46 ± 0.13	3.30 ± 0.37
	30 °C	3.36 ± 0.39	−5.05 ± 0.61	3.25 ± 0.05	2.70 ± 0.26
	35 °C	2.74 ± 0.10	−3.49 ± 0.16	2.52 ± 0.13	2.23 ± 0.99
Thyme 1	25 °C	3.69 ± 0.12	−6.05 ± 0.18	3.38 ± 0.16	2.99 ± 0.12
	30 °C	3.16 ± 0.12	−4.44 ± 0.16	2.94 ± 0.12	2.59 ± 0.12
	35 °C	2.12 ± 0.42	−2.37 ± 0.60	1.73 ± 0.04	1.74 ± 0.33
Thousand Flowers 1	25 °C	6.10 ± 0.73	−10.55 ± 0.89	5.60 ± 0.49	4.77 ± 0.22
	30 °C	3.93 ± 0.52	−6.34 ± 0.80	3.19 ± 0.26	3.24 ± 0.40
	35 °C	2.78 ± 0.35	−3.90 ± 0.63	2.62 ± 0.33	2.31 ± 0.28
Eucalyptus 1	25 °C	5.24 ± 0.25	−9.43 ± 0.44	4.78 ± 0.21	4.11 ± 0.17
	30 °C	4.23 ± 0.32	−6.63 ± 0.42	3.95 ± 0.30	3.38 ± 0.27
	35 °C	2.64 ± 0.07	−3.60 ± 0.13	2.46 ± 0.53	2.18 ± 0.05
Heather 2	25 °C	4.07 ± 0.30	−6.56 ± 0.26	3.71 ± 0.31	3.26 ± 0.24
	30 °C	2.78 ± 0.47	−3.94 ± 0.81	2.53 ± 0.41	2.25 ± 0.31
	35 °C	1.98 ± 0.17	−2.27 ± 0.20	1.76 ± 0.13	1.56 ± 0.93
Thyme 2	25 °C	4.11 ± 0.35	−4.77 ± 0.56	3.62 ± 0.33	3.16 ± 0.33
	30 °C	3.07 ± 0.76	−4.10 ± 0.93	2.40 ± 0.23	2.48 ± 0.59
	35 °C	2.00 ± 0.17	−2.14 ± 0.27	1.83 ± 0.16	1.64 ± 0.13
Thousand Flowers 2	25 °C	5.72 ± 0.57	−9.87 ± 0.86	5.20 ± 0.52	4.43 ± 0.40
	30 °C	3.28 ± 0.04	−5.19 ± 0.66	2.94 ± 0.01	2.61 ± 0.02
	35 °C	2.56 ± 0.07	−3.56 ± 0.80	2.45 ± 0.05	2.17 ± 0.02
Eucalyptus 2	25 °C	6.86 ± 1.76	−12.18 ± 1.98	5.68 ± 1.41	6.02 ± 0.98
	30 °C	4.34 ± 0.82	−6.47 ± 1.02	3.56 ± 0.32	3.56 ± 0.79
	35 °C	3.14 ± 0.54	−5.19 ± 0.14	2.95 ± 0.50	2.57 ± 0.41

Another parameter considered was the ultrasound pulse attenuation. This parameter, as mentioned above, is a measure of the progressive energy loss undergone by the signals as they propagate through the material. This loss is mainly due to two mechanisms: energy absorption (above all, thermoelastic effects) and scattering (reflection of the pulse front at discontinuities in the material) [20]. Attenuation is usually measured from the echoes observed on the A-scan which originate from reflections in the material. Thus, the attenuation coefficient α (in neper/m) was computed as:

$$\propto = \frac{1}{2d}\ln\left(\frac{A_1}{A_2}\right)$$

where A_1 and A_2 are the (peak-to-peak) amplitudes of echoes 1 and 2, respectively, and $2d$ represents the space covered by the ultrasound wave between them.

3.2. Texture analysis

For this determination, samples at 25 °C, 30 °C, and 35 °C were analysed, since these temperatures are usually apply for honey storing. The texture analysis was carried out using a Texture Profile Analysis (TPA). The instrument used was a Texture Analyser TA XT Plus (Stable Micro Systems Ltd., Surrey, UK) with a cylindrical probe 5 cm in diameter. Samples were put into jars with a larger diameter (7 cm). These jars were filled to reach 2.5 cm in height at a depth of 1.25 cm, the filling rate being 150 mm/min, thus following the procedure carried out by Oroian et al. [21]. The parameters calculated were: hardness (N) maximum force required to compress the sample (peak force during the first compression cycle); adhesiveness (N × s), work necessary to pull the compressing plunger away from the sample; gumminess (N), work needed to chew a semi-solid sample to a steady state of swallowing (hardness × cohesiveness, the last one being the extent to which the sample could be deformed before rupture); chewiness (N), work needed to chew a solid sample to a steady state of swallowing (hardness × cohesiveness × springiness, the last one being the height that the sample recovers during the time that elapses between the end of the first compression and the start of the second). The present study was carried out in triplicate at the three aforementioned temperatures: 25 °C, 30 °C, and 35 °C.

Principal components analysis (PCA) was applied on TPA data, including all measured samples, by using the XLSTAT software (Addinsoft Pearson Edition 2014, Addinsoft, Paris, France).

4. Results and discussion

4.1. Longitudinal propagation speed

Fig. 5 shows the values of UPV_L obtained in the honey samples corresponding to both batches. They include the results obtained for the inspections carried out at the different temperatures used in the study with the Panametrics V318-SU transducer. Comparing these results with the literature, we observed that our data are quite consistent with other studies. An example is that by Singh et al. [9] which determined that the wave propagation speed in honey was between 1933 and 2031 m/s depending on the amount of sugar in the samples. Other studies [22] also reported very similar results to those of our samples, with an average value of 2030 m/s for honey in general. However, not all the comparisons are so consistent. Ratajski et al. [10] obtained values between 1690 and 1750 m/s in two types of honey: Oil-Seed-Rape honey, and an equal mix of Buckwheat and Multifloral honeys. The difference between their values and ours is largely due to the fact that the honeys came from different sources, in addition to their using a 2 MHz transducer. Our results are also very different from those reported by Oroian [11] who obtained higher speeds (ranged between 4980 and 5095 m/s) in honey varieties from Romania.

Fig. 5 shows the evolution of the propagation speed during the aforementioned cooling process for both batches and the four varieties of honey. The tendency of the speed of propagation of the wave to slow as the honey warms can be seen. This tendency has also been observed by other workers [10,11]. The ultrasound speed could indeed be considered a parameter of interest for discerning between different types of

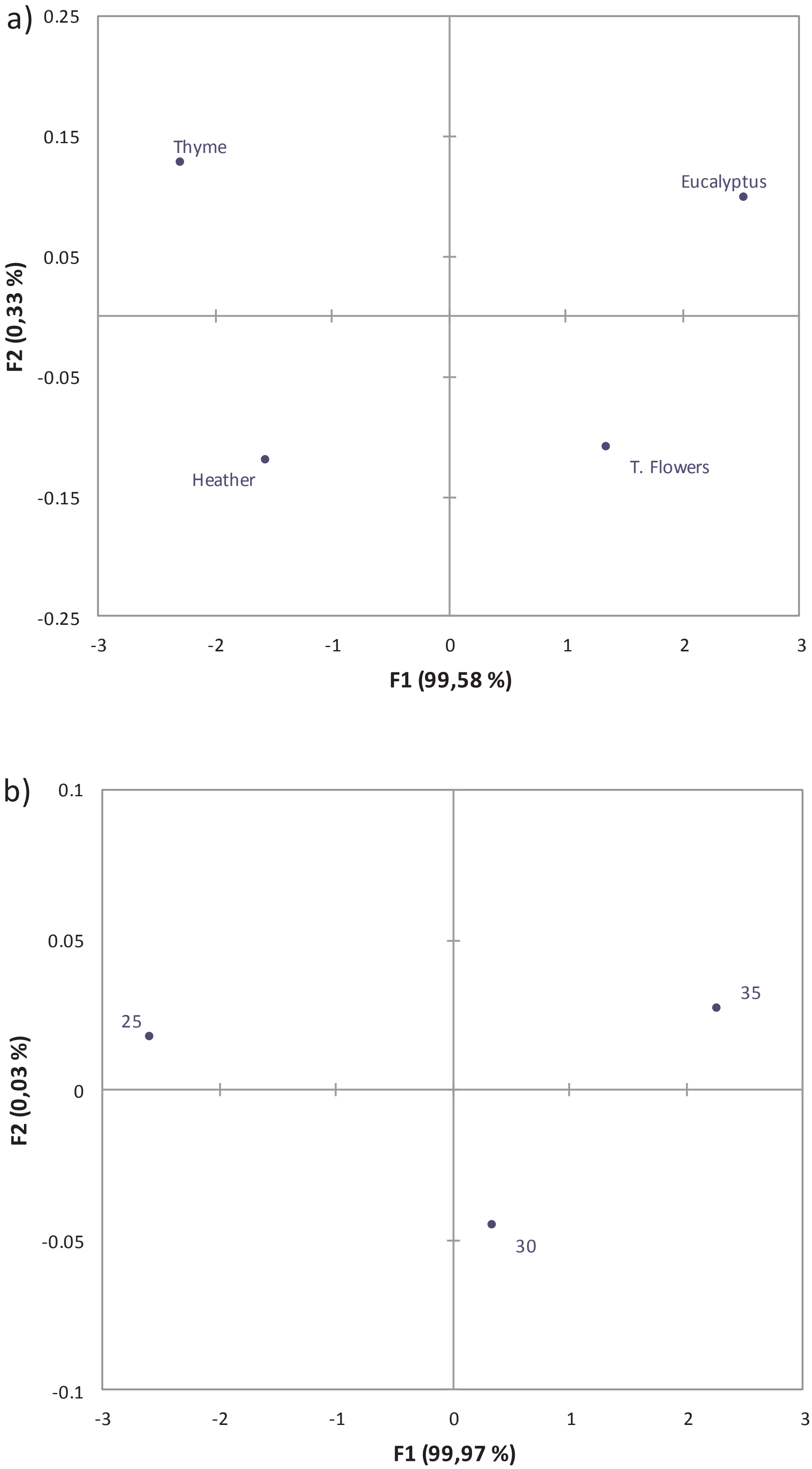

Fig. 7. PCA of TPA results (Hardness, Adhesiveness, Gumminess, and Chewiness) with factor score plots for variety (a) and temperature (b).

Table 3
Coefficients of linear correlations between the ultrasound and the texture parameters of the honeys.

		Hardness (N)	Adhesiveness (N × s)	Gumminess (N)	Chewiness (N)
UPV_L (m/s)	25 °C	0.71	−0.67	0.81	0.59
	30 °C	0.60	−0.75	0.54	0.60
	35 °C	0.65	−0.70	0.67	0.70
Attenuation (Np/m)	25 °C	0.24	−0.37	0.22	0.23
	30 °C	−0.55	0.44	−0.32	−0.57
	35 °C	−0.31	0.26	−0.17	−0.24

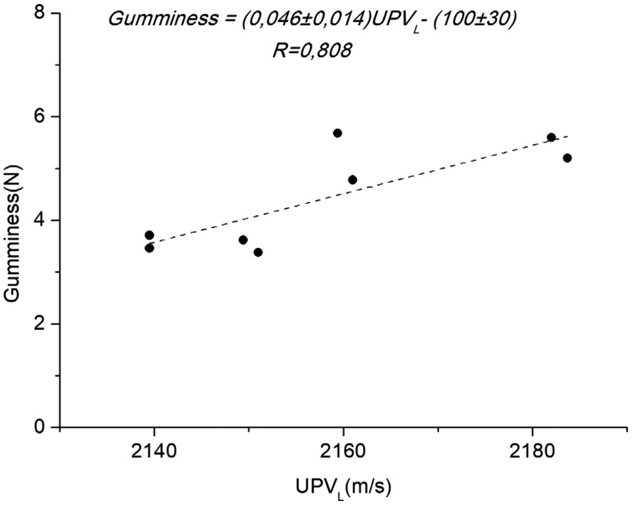

Fig. 8. Linear regression of the Gumminess with UPV_L measured at 25 °C.

honey. Thus, the separation is obvious between the different varieties. As seen in Fig. 5, the honeys form two groups. One comprises the samples of Thousand Flower and Eucalyptus, and the other the Thyme and Heather honeys. In any case, the speed of the ultrasound waves is a parameter that allows one to discern between the Heather and the Thousand Flower varieties, for example.

4.2. Attenuation

Fig. 6 shows the attenuation versus temperature for the two batches. The behaviour differs from the tendency exhibited by the UPV_L values. It must first be noted that attenuation was not determined in the Thousand Flower variety samples because the second echo signal was drowned out in the A-scan signal. Far from being a problem in assessing the samples, however, this clearly shows that the most attenuating samples correspond to the Thousand Flower variety.

One does not see any clearly increasing or decreasing tendency in the attenuation with temperature. The values of each sample are more or less stable, with only the Heather honey showing a clear rise in attenuation with temperature. Therefore, studying attenuation would allow the Thyme and Heather varieties to be distinguished which, until now, had practically been twins for the parameters linked to UPV_L.

It is thus evident that the conjoint study of all the ultrasound parameters considered in this research has allowed each of the honey varieties analysed to be characterized and classified unequivocally.

4.3. Texture profile analysis (TPA) results

Table 2 gives the mean values and standard deviations of the triplicate TPA results at three temperatures (25 °C, 30 °C, and 35 °C), in both batches, for the four varieties of honey. In general, texture parameters on honey decrease as temperature increase. These results closely resemble those presented by Oroian et al. [11,21] who found a negative influence of temperature on parameters of TPA. Hence, the likeliest explanation of these results is, as has already been proposed by other authors [21,23] that, as the temperature rises, the increased contact between the molecules of the honey causes the intermolecular forces, and hence also the textural parameters, to decrease. The ability of TPA for discriminate between honey samples depending on temperature and variety of honey was evaluated by PCA (Fig. 7a and b). As can be observed, a clear separation among samples has been found, indicating the accuracy of the instrumental texture parameters (hardness, adhesiveness, gumminess, and chewiness) for differentiating between honey samples as a function of variety and storing temperature. In addition, high correlation between texture parameters has also been found: hardness with adhesiveness (r = 0.984), hardness with gumminess (r = 0.986), hardness with chewiness (r = 0.992), adhesiveness with gumminess (r = 0.977), adhesiveness with chewiness (r = 0.981), and gumminess with chewiness (r = 0.970), hence, the study of texture parameters in honey could be simpler, by evaluating only one of them.

4.4. Correlation study

In this subsection, we shall describe the results of analysis of linear statistical correlations, of particular interest being some parameters that had not as yet been considered in other studies on honey. Table 3 presents the statistical correlations found between the ultrasound parameters and the texture at different temperatures. As can be seen, the strongest correlations were with UPV_L parameter. On the contrary, the attenuation data did not seem to be clearly correlated with the texture results. By way of example, Fig. 8 shows the correlation of UPV_L with gumminess at 25 °C.

The parameter that presented most correlations with the texture values was UPV_L. It is clear that the different responses of honey to the force or energy needed to deform or disintegrate it into an optimal state for deglutition clearly affect the speed of transmission of the wave through the sample. The parameters hardness, gumminess, and chewiness were positively correlated with UPV_L. This result was logical in the sense that these three texture parameters are linked to the force, energy, and work, respectively, applied to deform the honey or overcome its resistance to chewing. On the contrary, adhesiveness was negatively correlated with UPV_L, a logical consequence of the concept of adherence being linked to the work required to separate the surface of the honey from some other surface (tongue, teeth). The different consistency of the honey at the selected temperatures, thicker in the samples at 25 °C than at 30 °C, and thicker at 30 °C than at 35 °C, explain these results due to honey's greater fluidity as it gets warmer. This phenomenon has been noted by other workers who have observed a decrease in the coefficient of consistency as the temperature rises [24]. Likewise, other studies have shown that the textural parameter viscosity is significantly influenced by the temperature of honey samples and the concentration of soluble solids in them [23,25] with the latter being very important in the speed of transmission of the ultrasound wave, as was indicated above. Since the glucose and maltose contents of a honey, together with the moisture content, are linked to its textural parameters, our results are particularly interesting when it comes to characterizing different varieties by ultrasound.

5. Conclusions

The UPV_L results for the honey samples used in the present work were consistent with the most of values found in the literature. Increasing temperature of the honey samples led to a decrease in the speed of propagation of ultrasound waves. Only the Heather variety showed a clear increase in attenuation with temperature.

The conjoint study of the different ultrasound parameters (UPV_L and attenuation) at different temperatures made it possible to classify the different varieties of honey. In particular: (i) the UPV_L established two groups – Thousand Flower-Eucalyptus and Thyme-Heather; and (ii) attenuation separated both the Thousand Flower variety from the rest and the Heather variety from the rest. The TPA carried out also allows discerning clearly between the four varieties of honeys analysed. Thus, the destructive nature and the high variability of the data in texture analysis makes ultrasonic inspection more suitable, a priori, in this kind of analysis.

Moreover, the statistically significant correlations found between the ultrasound and textural parameters of the four honey samples add value to the utility of this non-destructive technique. These correlations lend further support to the use of ultrasound as a non-destructive technique for the characterization of honey, since they are properties that can directly contribute to assessing the incidence of the sugars and moisture content of a honey on its acoustic properties.

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