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Non-invasive and non-destructive ultrasonic technique for the detection of microbial contamination in packed UHT milk

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Abstract

An eight-channel ultrasonic device was developed to detect microorganism growth in UHT milk contained in carton-like packages without opening the packs. The system analyses automatically the amplitude and the delay of an ultrasonic pulse passing through packed UHT processed milk, being the coupling between the transducers and the packs accomplished in dry conditions. Changes in these parameters produced by different microbes are detected even when other physical–chemical parameters still remain within the sterility margins. Three different strains (*Bacillus cereus*, *Proteus vulgaris* and *Bacillus pumilus*) were inoculated at different concentrations in UHT milk packs. Variations in the velocity and amplitude of the ultrasonic wave show the growth signature of these microorganisms. Growth detection was achieved between 7 and 48 h depending on the number and type of bacteria inoculated. The experiments show that conventional analysis like pH or acidity measurements could be substituted by this non-invasive technique.

Keywords: Milk packs; Microbiological quality; Microorganism detection; Non-invasive measurement; Ultrasound

1. Introduction

The quality control assessment is a matter of prime interest for food producers. The society is becoming more and more sensitive about the quality of the food which is consumed. Therefore, a great attention is paid to the development of new sensors to carry out the quality control demanded by the industry. As a consequence, emerging biological, chemical and physical sensing technologies are topics of an intense research.

Microbiological quality assessment is an important stage of the production chain for many foodstuffs for which biological contaminations would produce a severe negative effect over consumers. A fraction of the foodstuffs emerging from the production line is used for sterility evaluation while the rest of the stock is retained until the microbiological quality of the tested samples is assured. Since some decades, new methods based on impedance or colorimetric measurements, ATP detection or cytometry are replacing the more traditional ones like pH and acidity measurement or direct total viable microorganism count. The trend of these new developments is towards time assay reduction, sensitivity improvement and procedure automation to diminish the storing requirements and contaminated product wastes.

The time taken to complete a microbiological test depends on the time taken for microbes to grow enough to reach the detection threshold of the method used. Thus, a previous incubation stage is performed to increase as

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much as possible the hypothetical sample microbial load. This stage must be long enough to let slowly growing microorganism multiplication. Microbiological control protocols in factories must take into account this stage which is function of the nature of the foodstuff and the potential contaminations. No matter how fast the microorganisms multiply, the contamination would not be detected until the incubation time is over and the contaminated sample is taken for analysis. The food produced at the line since the contamination started may be contaminated too, with potentially huge industrial losses. A technique able to analyse non destructively the foods since the incubation starts would allow the microbial detection as soon as the sensitivity thresholds of the method are reached, saving time, money and decreasing environmental hazards due to the microbiological contamination growth in the product.

Ultrasonic testing has been widely used from many decades for the quality control of many manufactured products, especially in the engineering area. It is straightforward that the advantages of this technology could be of interest for the food industry also. These techniques have the important features of being potentially non-invasive, non-destructive and suitable for opaque media analysis, as it is the case of some foods and packages. Finally, another important advantage of this technology is that ultrasonic low intensity mechanical waves are completely innocuous for the human health.

For these reasons, ultrasonic based instrumentation is increasingly being used in the food and feed industry quality controls (Denbow, 2001; Kress-Rogers, 2001). Some of these systems have the same function as they have in other industries, for example in fluid flow velocity measurement, vessel level control (either for liquid and solids), object presence detection or object count. In addition, new ultrasonic inspection methods have been developed for specific functions (McClements, 1995), for example, characterisation of food dispersions (Povey, 1999), composition measurement in milk (Miles, Shore, & Langley, 1990) and juices (Contreras Montes de Oca, Farley, McClements, & Povey, 1992), milk coagulation monitoring (Bakkali et al., 2001) or cheese manufacturing quality control (Benedito, Carcel, González, & Mulet, 2002).

Ultrasonic technologies have been already used for food microbiological detection. Nagata, Kaneoka, Imano, and Matumoto (1987) patented an ultrasonic inspection method for liquid foods based on the measurement of the speed and attenuation of an ultrasonic pulse travelling through a submerged bottle. Medical technologies were applied to detect milk spoilage by means of an echographic imaging system (Ahvenainen, Mattila, & Wirtanen, 1989) or a doppler ultrasonic technique (Gestrelius, Mattila-Sandholm, & Ahvenainen, 1994) to measure the acoustic streaming induced by ultrasound. Nevertheless, the measurement precision reached in the cited works was not enough to detect the contamination before important changes were caused in the milk. Hæggström (1997) was capable of detecting contaminations at earlier growing stages using specially modified packs of milk and soup submerged in water. Recently, the authors of the present paper developed an ultrasonic device for non destructive microbiological growth detection inside milk carton packs (Elvira, Montero de Espinosa, Resa, & Gómez-Ullate, 2003) in dry coupling conditions.

The objective of this work is to analyse the performance of the ultrasonic device presented to achieve a rapid non-destructive microbial growth detection in packed UHT milk. For this purpose, milk packs were inoculated with different microorganisms and different "detection signatures" were obtained which can be clearly distinguished from the sterility signature. An evaluation of the microbial thermal stress produced by the milk sterilisation process over the detection time is also presented.

2. Materials and methods

2.1. Ultrasonic device for microbial detection in packed liquid foods

The ultrasonic device for microbial detection was described in detail in previous papers (Montero et al., 2003; Elvira et al., 2003). It mainly consists of a chamber equipped with temperature and humidity control systems. Eight milk packs can be tested simultaneously. They are placed in independent housings. A pair of piezoelectric transducers working on through-transmission are coupled face to face to the sides of each pack. The active element of these transducers is a 1 MHz, 20 mm diameter piezoceramic disc (Ferroperm PZ27). An ultrasonic burst with a central frequency of 800 kHz emitted by one transducer is received by the other one after travelling through the milk package.

The sample temperature is increased from the temperature at packaging (~27 °C) up to 35 °C and maintained with a ±0.01 °C stability during the measuring process. The amplitude and the time-of-flight taken by the signal to reach the receiver is measured for each pack in 2 min intervals. Variations of these ultrasonic parameters related to physical–chemical changes of the milk, respect to the traces obtained by measuring sterile milk, may reveal the growth of a microorganism.

2.2. Sample inoculation

Three different strains from the Colección Española de Cultivos Tipo, were used for the tests: *Bacillus cereus* (CECT-148), *Proteus vulgaris* (CECT-484) and *Bacillus pumilus* (CECT-29).

The bacterial culture preparation for milk inoculation was as follows. The strain was initially lyophilised inside a glass vial, which is cut in a laminar flux cabin (Telstar, Micrón-V). 0.2 cc of buffered peptone water (Merck Cod.107228) was added and the resulting suspension was carefully mixed to avoid bubble formation. From this, a 0.1 cc aliquot was dispensed into 150 cc of buffered peptone water. The new suspension was incubated during 36 h at 32 °C ± 0.2 in a culture oven (Selecta Gc.102). After incubation, total viable aerobic bacteria was determined using Plate Count Agar, PCA (Bioser cod. BK 144 HA). The procedure was repeated, dispensing an aliquot of this suspension into 150 cc of buffered peptone water which was incubated again for 36 h at 32 °C. From the bacterial culture an aliquot was dispensed in ringer solution (Oxoid, BR00526). Two different dilutions were prepared for each test. 1 cc samples of each suspension were used to determine the total viable bacteria inoculated by PCA. In each test, three packs were inoculated with 1 cc of one dilution, another three packs with 1 cc of the second dilution and two packs were left sterile. The ringer solution volume of these dilutions were changed for different tests to analyse the performance of the ultrasonic detection system for initial microbial concentrations between 10^{-3} and 10^{2} cfu/g. The lowest concentration corresponds to the case in which only one bacteria was initially present in the pack.

Pack inoculation is critical in the experimental procedure because the holes made in the packs could allow the milk entering the carton layer, causing an irreversible damage on it and, sometimes, external contamination. The milk pack is placed into a stainless steal specially adapted holder, which makes some gentle pressure on it, leaving free a zone to make a hole with the inoculation syringe. The holder inclination prevents the milk coming out from the hole and so, no milk penetrates in the carton layer. The pressure assures that, when the hole is made, exterior air does not enter the pack. The place where the hole is made is previously sterilised using Ethanol 96° (Panreac Química S.A., cod. 121085.1612). After inoculation, a drop of rapid cyanocrylate adhesive is applied to the hole penetrating a little bit in the carton layer sealing it to avoid milk wetting. Finally a thermoplastic bonding agent is used to close and protect the hole.

2.3. Incubation conditions

After inoculation, the milk packages were placed in the ultrasonic detection device housings. The incubation temperature was set at 35 °C, because the growing of many microorganisms, which are relevant for milk production, is enhanced at this temperature. It takes between 1 and 2 h to rise the milk temperature from the 27 °C reached after packing to the 35 °C desired for incubation. The chamber humidity was set to 55% that was found the best value to diminish as much as possible the wet interchange between the packs and the ambient. Due to the constraints imposed by the inoculation which deteriorates the isolation properties of the packages, 4–6 h was needed to reach the humidity stabilisation in all packs.

The ultrasonic analysis is made while the incubation of the packs is made, which means that the standard temperature and time protocols during the ultrasonic measurements remain unaltered.

2.4. Post-incubation analysis

After the ultrasonic analysis of the packs is performed, microbiological and physical–chemical evaluation of the inoculated milk was done.

The zone were the packs are opened is sterilised with ethanol 96 °C. A milk sample is diluted in ringer and plated on PCA. After incubating the plates for 24–48 h at 32 °C, the total viable count is obtained. The physical–chemical tests include:

- pH measurement with pH meter WTW 330i.
- Acidity measurement, to evaluate the organic acids present in the milk. NaOH N/9 (Merck cod.B191998 230) was suspended in a 10 cc milk sample with a fenolftaleine 1% solution in Ethanol 96 °C (Panreac Quimica S.A. cod.131325).
- Stability evaluation. Two 1 cc milk samples are suspended in 1 cc of Ethanol 80° and 1 cc of Ethanol 90°, respectively. The floc formation is observed.

3. Results and discussion

3.1. Contamination detection traces

As it was said, physical–chemical milk changes produced by a growing microorganism result in trace deviations from the sterile behaviour. Trace variations corresponding to the amplitude and the time-of-flight of the ultrasonic pulse during the first 5 h are due to the increasing of the milk and pack temperature, stabilisation of the pack carton layer humidity and stabilisation of the physical–chemical properties of the milk after the UHT process (see Fig. 1). Within this time, no information about the sterility conditions of the milk can be obtained. The amplitude was normalised to the signal amplitude value obtained at that fifth hour. Nevertheless, from the measurements obtained between hours 5 and 7, the sterile behaviour of the rest of the trace can be calculated reasonably well (Fig. 1(a) and (b)).

It was expected that the time-of-flight curves after the fifth hour would be horizontal. However, defects in the hole sealing make the humidity slowly increase with the result of an almost straight increasing of the time-of-flight. These traces, which follow a straight behaviour,

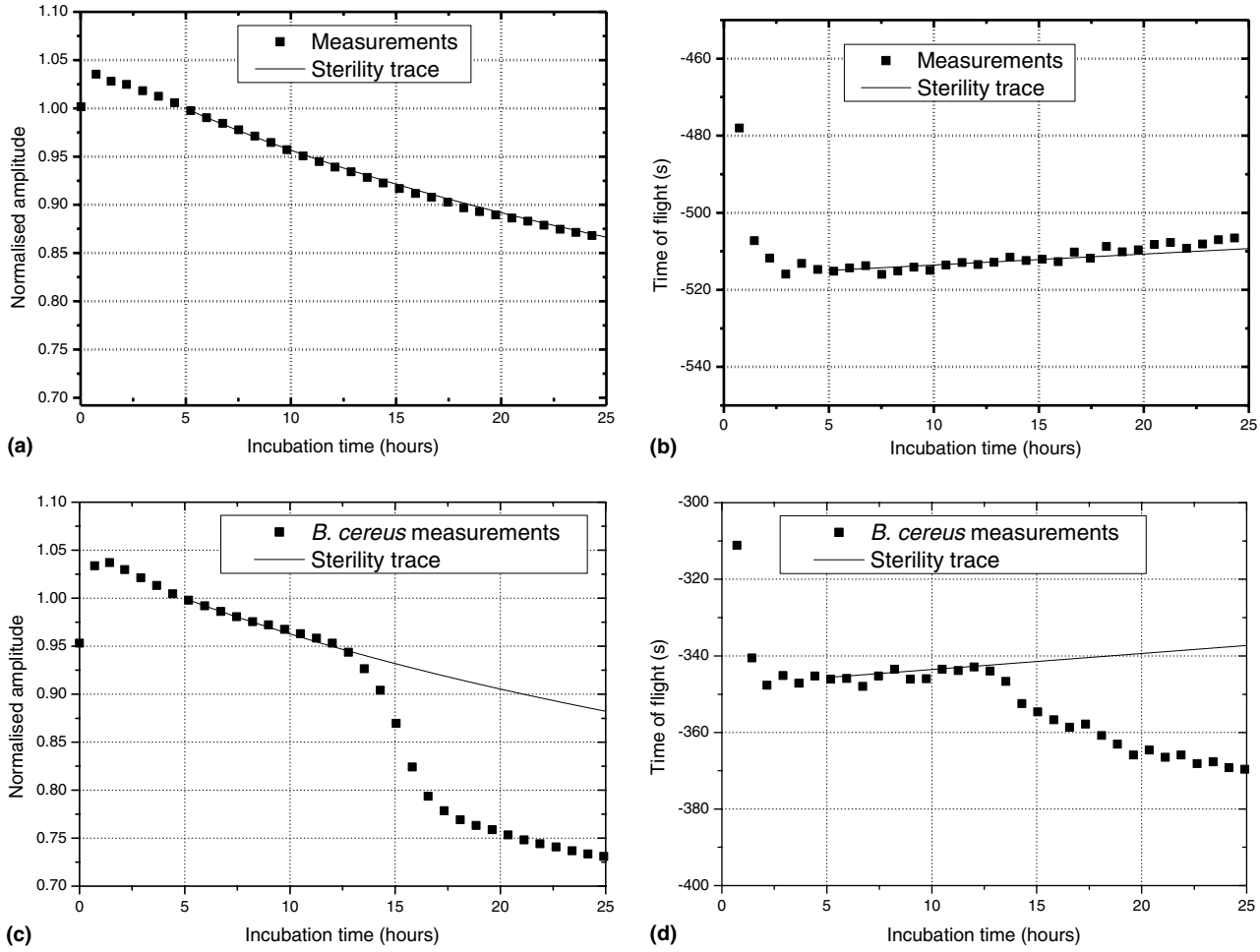

Fig. 1. Normalised amplitude (a, c, e and g) and time-of-flight (b, d, f and h) measurements –squares– of UHT milk packs. The continuous line is the sterility trace obtained from the measurement between hours 5 and 7. (a) and (b) correspond to sterile milk, (c) and (d) correspond to sterile milk inoculated with *B. cereus*, (e) and (f) correspond to sterile milk inoculated with *B. pumilus* and (e) and (f) correspond to sterile milk inoculated with *P. vulgaris*.

take this effect into account showing a positive slope, as can be seen in Fig. 1(d), (f) and (h).

Fig. 1(c)–(h) show the ultrasonic measurements and sterility curves obtained for three different tests of *B. cereus*, *B. pumilus* and *P. vulgaris*. Post-incubation analysis corresponding to these assays are written in Table 1. The deviation from the sterile behaviour can be seen in all plots except in Fig. 1(f) that shows the time-of-flight measurement of milk inoculated with *B. pumilus*. No effect over this parameter was detected, perhaps because this contamination produces small changes in the physical–chemical parameters shown in Table 1, which fall within the sterility range. On the contrary, the ultrasonic amplitude (Fig. 1(e)) decreases clearly, although slower than *B. cereus* contamination curve (Fig. 1(c)), which makes possible the ultrasonic detection of *B. pumilus*.

These results seem to state a relation between the physical–chemical parameters and the time-of-flight measurements. Nevertheless, this relation may be affected by other mechanism because the pH and the acidity values for *B. cereus* and *P. vulgaris* at detection (Table 2) show a lower change in the case of the *B. cereus*. Looking at the amplitude, it was found a decreasing of it for both *Bacillus* and an increasing for *P. vulgaris*. The appearing of an increasing concentration of bacteria could rise the viscous attenuation, but the behaviour of the amplitude measurements in the case of *P. vulgaris* points to another factor as relevant for the acoustic amplitude. Probably this parameter is mainly affected by the particle distribution in the milk which depends on the protein distribution (with medium initial sizes under 0.1 μm) and the lipid distribution (with medium initial sizes near 5 μm). In addition, the different metabolism of the microorganisms could give different ultrasonic amplitude variations. It is known that the decreasing of pH by the lactic acid produced during the lactose fermentation induces the protein coagulation which affects the amplitude (and also the speed) of an ultrasonic wave propagating through that media. A

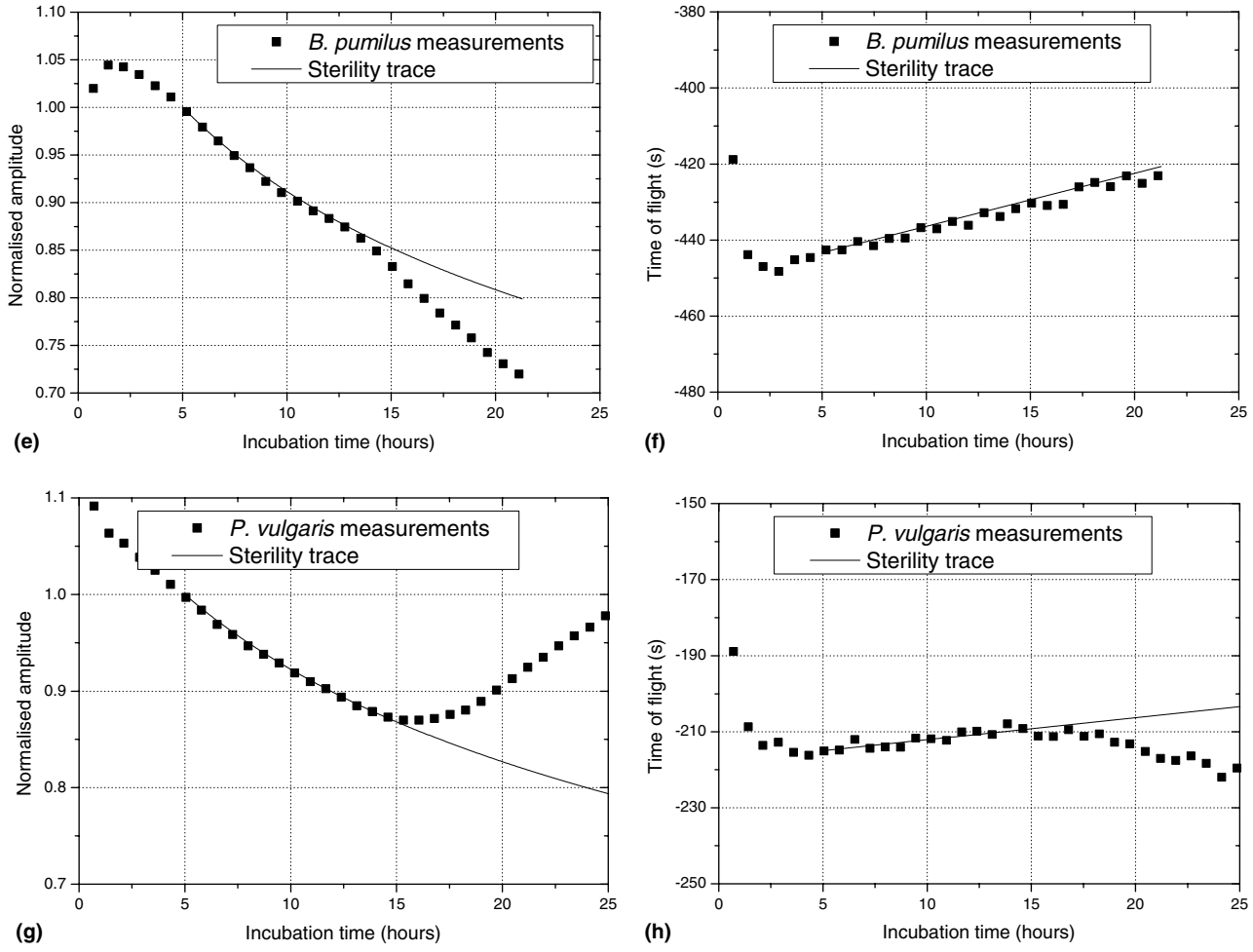

Fig 1. (*continued*)

Table 1
Physical–chemical and microbiological tests after 25 h of incubation

Microorganism	Initial (cfu/g)	Final (cfu/g)	pH	Acidity (°*D*)	Stability[a] (80/90)
Ø (sterile)	0	0	6.65 ± 0.05	14.0 ± 0.5	+/+ or −
B. cereus	0.01	$4.3 * 10^7$	6.38	20.7	−/−
B. pumilus	0.80	$1.8 * 10^7$	6.62	14.3	+/−
P. vulgaris	0.95	$2.6 * 10^8$	6.34	17.4	−/−

[a] Signs + and − refer to stability and instability, respectively.

Table 2
Physical–chemical and microbiological tests at ultrasonic detection

Microorganism	Final (cfu/g)	pH	Acidity (°*D*)	Stability[a] (80/90)
Ø (sterile)	0	6.65 ± 0.05	14.0 ± 0.5	+/+ or −
B. cereus	$2.6 * 10^7 \pm 1.1 * 10^7$	6.49 ± 0.05	15.3 ± 0.6	−/−
B. pumilus	$1.6 * 10^7 \pm 0.8 * 10^7$	6.64 ± 0.05	14.4 ± 0.5	+/−
P. vulgaris	$2.5 * 10^8 \pm 1.3 * 10^8$	6.38 ± 0.11	17.1 ± 1.6	−/−

[a] Signs + and − refer to stability and instability, respectively.

more precise analysis of the acoustic behaviour in contaminated milk would rely upon a detailed analysis of the metabolism of different contaminant bacteria and their effect over the milk particle distribution.

3.2. Concentration thresholds for microbial detection

A mathematical algorithm was developed to detect any deviation of the ultrasonic measurements from the

sterile behaviour, activating an alarm when this happens. A set of tests was done to analyse the physical–chemical properties and the microbial concentration in the milk when this alarm was activated for the different microorganisms. The results are displayed in Table 2. The lowest threshold was obtained for *B. pumilus*, being only slightly lower than the *B. cereus* threshold. One magnitude order bigger was the *P. vulgaris* threshold. These values are related to the microbial concentration needed to produce detectable physical–chemical changes in the milk and could be different for another media. The measurements of pH and acidity are similar to those shown in Table 1, although just when the ultrasonic detection take place (Table 2) they are closer to the values corresponding to sterility. It is interesting to note that the pH corresponding to sterility (6.65) falls into the error margins corresponding to the pH measured at the detection of the *B. pumilus* contamination (6.64 ± 0.04). No important growth of the colony takes place after the trace begins to deviate from the sterile behaviour. This implies that the ultrasonic measurement is affected basically by the changes in the medium caused by the bacterial metabolism (solved components, proteins and lipids), instead of the concentration change of the microorganisms.

In Fig. 2 the time needed for these microorganism to be detected as a function of the initial microbial charge is analysed. This time gives an idea of the maximum detection time expected for the strains tested. It was obtained from the ultrasonic amplitude measurements, because the time-of-flight did not give any growing indication for the *B. pumilus* case. Nevertheless, for the cases of *B. cereus* and *P. vulgaris*, both the amplitude and the time-of-flight could be used for the detection time analysis because, as it can be seen in plots Fig. 1(c), (d), (g), and (h), both acoustic parameters deviate from the sterility curves at the same time.

The dispersion of the obtained results was high, especially for the case of *B. pumilus* contamination. The minimum initial concentration analysed was 0.001 cfu/g, corresponding to the case in which only one microorganism enters into the milk pack. The minimum detection times were obtained for *B. cereus*, taking place 7 h after the incubation began in the cases were the initial concentration was higher than 1 cfu/g. In all the tests done with this strain, the contamination was detected in less than 24 h, and the detection usually takes place between 7 and 15 h. The detection times, were higher for *B. pumilus* and *P. vulgaris* (usually within 12–30 h). In Fig. 3, the three linear fits corresponding to curves plotted in Fig. 2, are shown together, and the regression coefficients are shown in Table 3. A similar slope in the case of both *Bacillus* (−1.62 and −1.79) was obtained, which seems to indicate that both microorganisms present a similar replication time. Nevertheless, *B. pumilus* may have a longer latency period before starting replication, which gives rise to that long detection time compared to *B. cereus*. The more negative slope shown by the *P. vulgaris* contamination (−2.63) could be related to a slower replication time and/or a different metabolism process producing slower changes in the ultrasonic parameters.

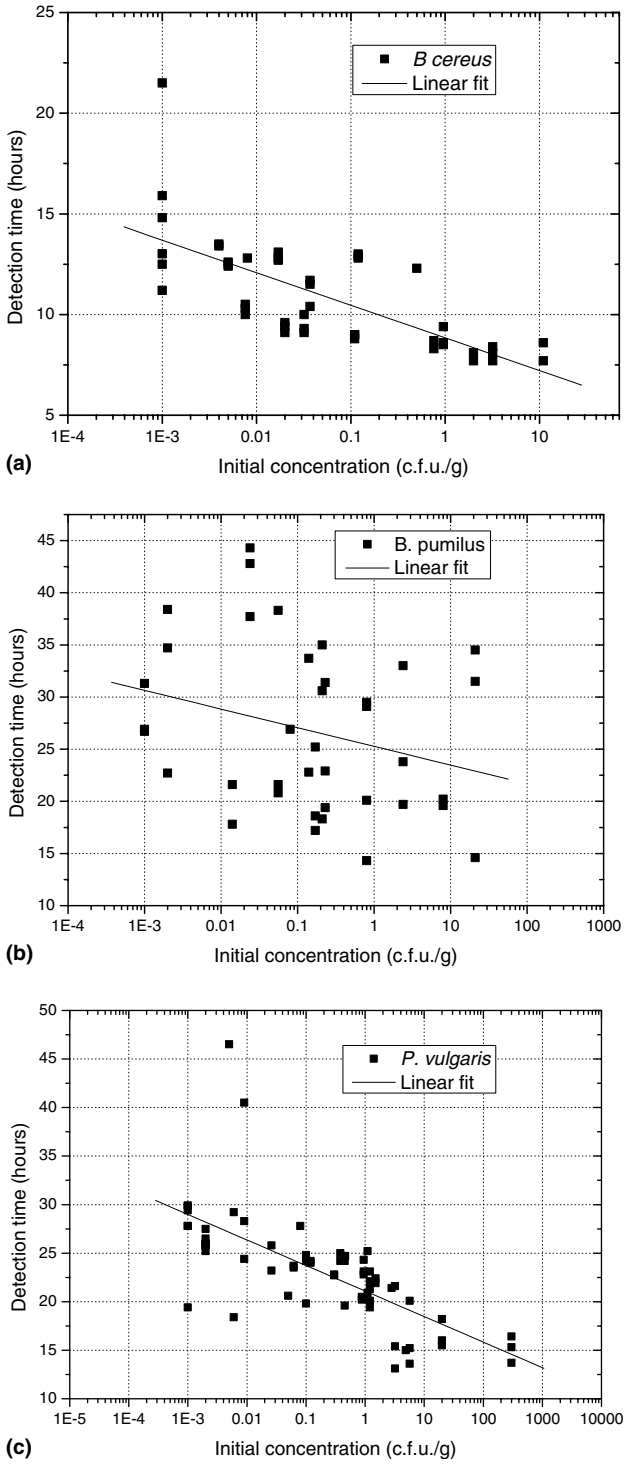

Fig. 2. Detection time (squares) of UHT sterile milk inoculated with different concentrations of *B. cereus* (a), *B. pumilus* (b) and *P. vulgaris* (c). The continuous line is the linear fit of the measurements obtained.

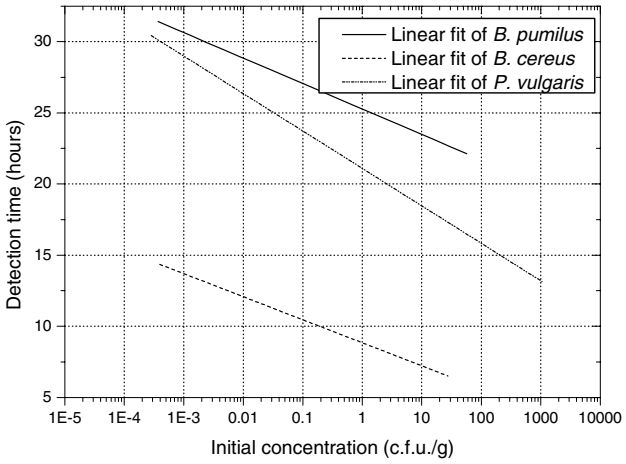

Fig. 3. Detection time linear fits for *B. pumilus* (continuous line), *P. vulgaris* (dashed line) and *B. cereus* (dashed-dotted line). The regression parameters are shown in Table 3.

Table 3
Regression parameters corresponding to detection times linear fits of Fig. 3

Microorganism	A (h g/cfu)	B (h)	R^2
B. cereus	−1.62	8.84	0.53
B. pumilus	−1.79	25.27	0.08
P. vulgaris	−2.63	21.09	0.48
P. vulgaris (heat treated)	−4.13	25.58	0.28

A and B correspond to the line equation: $y = Ax + B$, and R^2 is the coefficient of determination (square of the correlation coefficient).

3.3. Thermal stress influence over detection

Microorganisms surviving sterilisation processes or other kind of stresses may be hardly detected than laboratory prepared cultures. This effect was studied by thermally stressing the *P. vulgaris* culture. For that purpose and before inoculation, the culture was place in a thermostated bath at 50 °C for 5 min. This treatment causes a severe decreasing of the cfu concentration between 50% and 99.9%. The PCA control was done after the thermal treatment to compare the results with the non-stressed *P. vulgaris* case.

The measurements obtained are plotted in Fig. 4 (circles) and compared to the detection time obtained without heating the culture (squares). The dispersion of the results is even higher, but an increasing between 5 and 10 h in the detection time was measured. The stress applied with the five minutes treatment does not prevent from the ultrasonic contamination detection, but it can increase the time needed for it, giving a slower replication time as it can be deduced from the more negative slope (parameter A) shown in Table 3.

Longer time heating periods at the same temperature were applied and sometimes the complete elimination of viable bacteria was produced. In Fig. 5, the number of positive results obtained by PCA and ultrasonic measurement (before 48 h) for different thermal treatment times are displayed. From a total of 56 samples measured, there were five cases in which a negative ultrasonic result was follow by a positive PCA result (1 for 10 min, 3 for 17 min and 1 for 20 min of heating). In all these cases the cfu concentration measured after incubation was under the ultrasonic threshold for *P. vulgaris* shown in Table 2. Therefore, the thermal stress delayed the growth of the microorganisms, but in spite of this, the microorganisms can be detected by ultrasound if the incubation time is long enough to reach the concentration threshold of the bacteria. The pH, the acidity and the stability measured in the cases in which the ultrasonic detection was not achieved, do not reveal the presence of the contamination either.

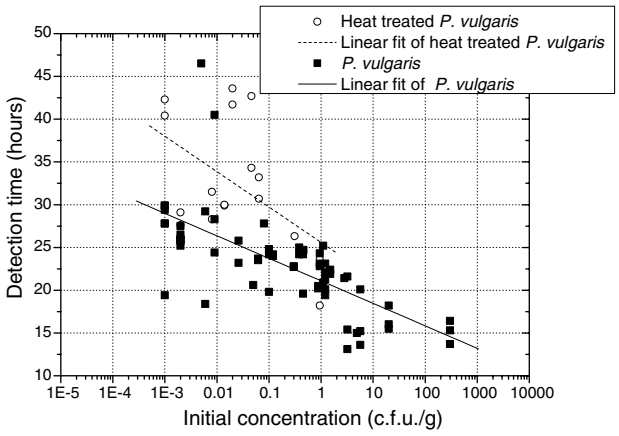

Fig. 4. Detection time of UHT sterile milk inoculated with different concentrations of non stressed *P. vulgaris* (squares) and thermally stressed *P. vulgaris* (circles). The continuous line and dashed line are the linear fits of the non stressed strain and the thermally stressed strain measurements, respectively. The regression parameters are shown in Table 3.

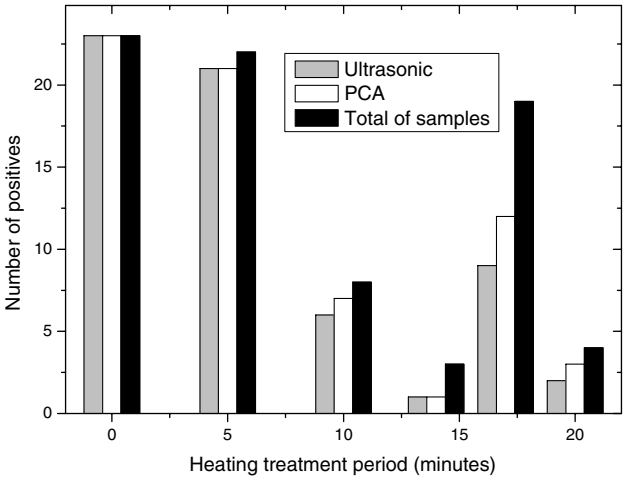

Fig. 5. Histogram showing the number of positive results obtained by PCA (white bar) and ultrasonic (grey bar) measurement (before 48 h) for different thermal treatment times. The black bars represent the total number of tests carried out.

4. Conclusion

The development of non-invasive and non-destructive methods for microbiological quality analysis of packed products, allows a reduction of the time needed to detect a given contamination. This is possible because the measurement is obtained in real-time during incubation and it is not necessary to wait the number of hours marked by the protocol to take a sample for control.

An ultrasonic device for the analysis of liquid packed foods was analysed in this paper. This device performs the incubation of the packs while taken continuous measurements of the ultrasonic propagation characteristics in the milk. The contamination growth in packed UHT milk inoculated with three different strains (*B. cereus*, *B. pumilus* and *P. vulgaris*) was studied. It was shown that some contaminations can be detected non-invasively at 7 h from the incubation beginning.

The evolution of the ultrasonic propagation velocity and amplitude when the microorganism tested grew in the milk was shown. Different patterns appear depending on the microorganism tested which indicates that their metabolism induce different changes in the milk. The ultrasonic results were compared with total viable bacteria count and physical–chemical parameters (pH, acidity and stability to ethanol). It was shown that ultrasonic detection could take place even before detectable physical–chemical changes appear. The cfu concentration thresholds for the ultrasonic detection of the strains analysed were established.

The influence of microbial thermal stress over the efficiency in the detection of *P. vulgaris* milk contamination was also analysed. The detection time increases although the concentration thresholds remains the same.

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References

Ahvenainen, R., Mattila, T., & Wirtanen, G. (1989). Ultrasound penetration through different packaging materials – a non-destructive method for quality control of packed UHT milk. *Lebensmittel-Wissenschaft & Technologie, 22*, 268–272.

Bakkali, F., Moudden, A., Faiz, B., Amghar, A., Maze, G., Montero de Espinosa, F., & Akhnak, M. (2001). *Measurement Science and Technology, 12*, 2154–2159.

Benedito, J., Carcel, J. A., González, R., & Mulet, A. (2002). Application of low intensity ultrasonics to cheese manufacturing proceses. *Ultrasonics, 40*, 19–23.

Contreras Montes de Oca, N. I., Farley, P., McClements, D. J., & Povey, M. J. W. (1992). Análisis of the sugar content of fruit juices and drinks using ultrasonic velocity measurements. *International Journal of Food Science Technology, 27*, 515–529.

Denbow, N. (2001). Ultrasonic instrumentation in the food industry. In E. Kress-Rogers & C. J. B. Brimelow (Eds.), *Instrumentation and sensors for the food industry* (2nd ed.) (pp. 326–354). Cambridge, England: Woodhead Publishing Limited.

Elvira, L., Montero de Espinosa, F., Resa, P. & Gómez-Ullate, Y. (2003). Detección y análisis no invasivo de microorganismos en productos lácteos envasados en recipientes laminados basados en papel o cartón utilizando ondas elásticas por acoplamiento en seco. Patent PCT/ES 03/00287.

Gestrelius, H., Mattila-Sandholm, T., & Ahvenainen, R. (1994). Methods for non-invasive sterility control in aseptically packaged foods. *Trends in Food Science and Technology, 5*(12), 379–383.

Hæggström, E. (1997). Ultrasound detection of microbe contamination in premade food. *Acta Polytechnica Scandinavica Applied Physics Series, 214*, 1–115.

Kress-Rogers, E. (2001). Ultrasound propagation in food and ambient gases: principles and applications. In E. Kress-Rogers & C. J. B. Brimelow (Eds.), *Instrumentation and sensors for the food industry* (2nd ed.) (pp. 355–402). Cambridge, England: Woodhead Publishing Limited.

McClements, D. J. (1995). Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science and Technology, 6*, 93–99.

Miles, C. A., Shore, D., & Langley, K. R. (1990). Attenuation of ultrasound in milks and creams. *Ultrasonics, 28*, 394–400.

Montero de Espinosa, F., Elvira, L., Gómez-Ullate, Y., Resa, P., Matesanz, J., Iglesias, J. R., Ron, A., & Echevarría, F. J. (2003). Industrial system to perform the microbiological control of UHT milk in carton-like packages by ultrasound. In *Proceedings of the IEEE international ultrasonic symposium, Hawaii*.

Nagata, M., Kaneoka, T., Imano, S. & Matumoto, H. (1987). Ultrasonic method of inspecting contents of a package and apparatus thereof. Fujimori Kogyo Co. Ltd.. EP269815.

Povey, M. J. W. (1999). Ultrasonic determination of the properties of food dispersions. *Seminars in Food Analysis, 4*(2), 95–111.