Proceedings of 20th International Congress on Acoustics, ICA 2010

23-27 August 2010, Sydney, Australia

Ultrasonic Food Quality Analyzer Based on Cylindrical Standing Waves

Aba Priev and Yechezkel Barenholz

Hebrew University, Hadassah Medical School, Jerusalem, Israel

PACS: 43.20.Hq, Ks; 43.25.Qp; 43.35.Yb, Zc

ABSTRACT

An innovative technology based on the use of ultrasonic cylindrical standing waves for continuous monitoring of quality of various liquid food products, such as milk, juice, beer, wine, and drinking water, is described. A proprietary unique feature of the developed ultrasonic analyzer is that it employs a combined mode of operation using both high-intensity (5 W/cm^2) waves for separation and concentration of the large particles (fat globules, bacteria, or yeasts) and low-intensity (0.5 W/cm^2) waves for compositional analysis. High accuracy for sound velocity (> 0.001%) and sound absorption (~0.5%) measurements and rapid testing time (5-20s) have been achieved. Comparative analyses of the ultrasonic method with standard reference techniques have produced linear calibration curves for major components, with correlation coefficients higher than 0.95. It is thus possible to monitor total protein and fat content, and somatic cell count in raw milk in cowsheds, or salinity, turbidity, specific gravity, and particles (bacteria) in drinking water directly. Advantages of the proposed technology include its reagent-free nature, no need for sample pretreatment, ease-of-use, and low cost.

INTRODUCTION

Ultrasonic properties are very sensitive to composition of liquid food products. The first applications of ultrasound for food quality control started 50 years ago for determining alcohol in beverages, and fat in milk. Now the list of applications for optimization of quality of food products comprises hundreds of items. Multiple informative ultrasonic parameters can be measured: sound velocity and its temperature dependence, absorption and its frequency dependence, acoustic impedance and scattering (Table 1).

Table 1. Ultrasonic characteristics of liquids

Ultrasonic parameters	Properties of liquid sample
Sound velocity U, and Acoustic impedance ρU	Compressibility K, Density ρ, and Temperature T.
Sound absorption α, Frequency dependence of absorption *d*α/*d*F, and Scattering	Size of the particles R, Relaxation processes (energy losses, aggregation or conformational transitions)
Temperature dependence of sound velocity and absorption, *d*U/*d*T, *d*α/*d*T	Amount of bound water and hydrophobic compounds

Currently available commercial ultrasonic devices for scientific applications, based on *plane* standing waves and produced by Sonas Technologies Ltd (Dublin, Ireland) or by TF Instruments GmbH, Heidelberg, Germany) allow one to measure all of these ultrasonic properties, but cost about $100,000 and may not be applicable for all food industry applications.

CYLINDRICAL ULTRASONIC RESONATORS

In this paper we discuss an emerging technique using *cylindrical* ultrasonic standing waves. The unique simplicity of the cylindrical-wave-based device derives from the construction of the ultrasonic measuring chamber. This is just a tube, typically 5-10mm long, excited at natural frequencies of radial oscillations of the liquid column filling the tube. Devices based on the standing cylindrical waves will be far superior to other ultrasonic measurement instumentation in respect to industrial requirements such as cost, precision, ease of use in flow-through systems, and measurement of small samples of a few microliters. Our group, in collaboration with Artann Laboratories (NJ, USA), has been developing a variety of cylindrical ultrasonic resonators for food industry applications that use radially oscillating PZT piezoceramic tubes or their segments [1]. We discuss mainly compositional analysis of food products by using low- and high-intensity cylindrical waves. Applications of other types of standing wave resonators were described recently in other reviews for food testing [2-4] as well as for large particle separation and manipulation [5, 6].

Open piezotube

The open piezotube has a single radially-polarized piezoceramic tube (Figure 1A). *Open piezotube* resonators are very effective for simple dip-in monitoring of one or two acoustic parameters; for example, sound velocity and absorption. A differential version of an ultrasonic sugar analyzer has one of the resonators filled with water and a second with juice or other beverage [7, 8]. The main problem with the *open piezotube* is the difficulty of maintaining a constant temperature due to its low thermal conductivity. The cylindrical measurement chamber of the *open piezotube* is optimal for on-line monitoring (5s) and for easy cleaning.

Composite piezotube

Most industrial applications need high thermal stability of the sample during its analysis. The *composite piezotube*, where a metal tube is covered by two or more segments of cylindrical piezoceramics (Figure 1B), has a high thermal stability. Such resonators have been used in a multi-parametric liquid analyzer, where a thermostability of 0.01°C is obtained in 20s. It is just a temperature slope from 20 to 30°C that enables specifically determining alcohol in wine and beer, or fat in milk. In addition, metal tubes of the *composite piezotubes* can be used as electrodes for conductivity measurements at various frequencies [9, 10].

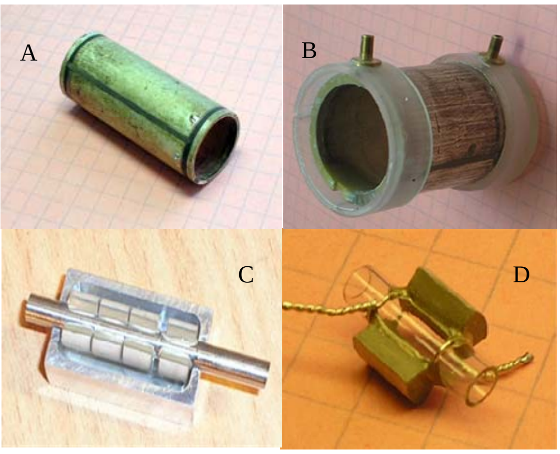

Figure 1. O*pen piezotubes* with a single radially-polarized piezoceramic tube (A, B). C*omposite piezotubes* with a metal (C) and a glass tube (B) covered by segments of cylindrical piezoceramics

Multiple sample components may be determined simultaneously by measuring more acoustical and physical properties of the sample. Thus, for example, in milk we have five main components: fat, protein, sugar, salt, and water, which can be measured directly at concentrations as low as 0.1%. By measuring at least five independent parameters, such as sound velocity, absorption, conductivity, and the temperature dependencies of sound velocity and absorption, one can get a single-valued solution of all equations and determine all main components of the milk in 20s (Table 2). In wine and beer this method allows one to test alcohol, sugar, extracts and specific gravity; and in water – salinity, turbidity, organics, and conductivity. The detection is fast, inexpensive, and requires as little as a drop of sample.

Table 2. Ultrasonic food product analysis

Food Product	Components of the Fluids			
Milk	Fat	Protein	Sugar	Salt
Wine, Beer	Alcohol	Sugar	Extracts	Specific Gravity
Water	Turbidity	Salinity	Organics	Gases

Closed piezotube

Figure 2 shows a third version of a cylindrical standing wave resonator, the *closed piezotube*. By limiting the ratio of length to radius of the tube of the resonator, one can significantly improve the quality factor and obtain high-resolution ultrasonic measurements. The *closed piezotube* includes non-interference bands formed by constrictions for isolating the area of the standing wave oscillations. Without such constrictions, waves traveling along the long axis would interfere with the standing waves within the resonator cavity and interfere with the clean separation of the large particles [11, 12]. In Figure 2 the resonance characteristics of the *open piezotube* and *closed piezotube* are compared.

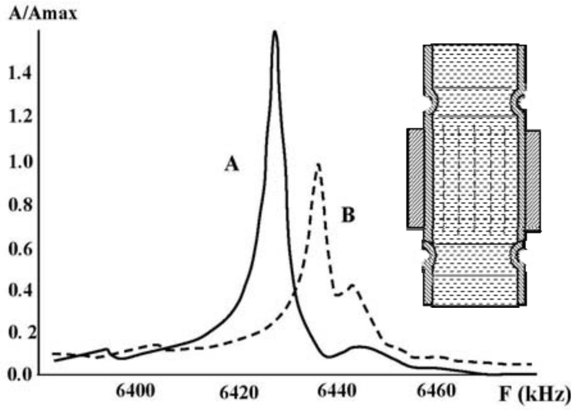

Figure 2. The resonance characteristics of this *closed piezotube* (A) in comparison to the *composite piezotube* (B). *At right* is a schematic view of the *closed piezotube*, having the limiting ratio tube length/radius of 1.5

The improved quality factor of the *closed piezotube* allows us to reach a higher intensity and to achieve a new feature of the cylindrical standing wave resonator: ability to concentrate and separate large particles (bacteria, cells, yeasts, fat globules) of the sample by the acoustic radiation forces. The cylindrical standing waves of high intensity create an acoustic radiation force that forms two areas of pressure, the node and anti-node, where the particles can be trapped and determined (Figure 3).

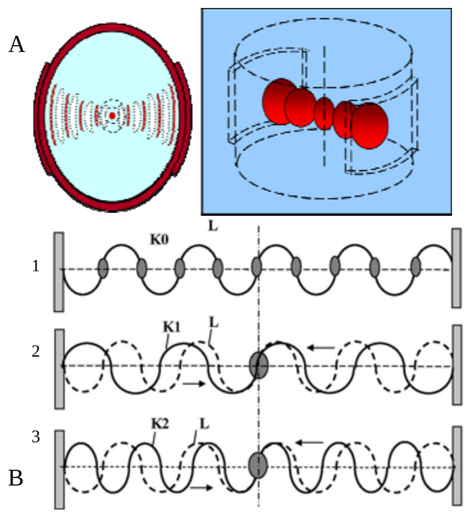

Figure 3. Node and anti-node areas of *closed piezotube* with two segments of piezoceramics (A) and the stages of concentrating of the large particles in the central node (B) [12]

It is important to mention that the process of concentrating of particles in the *closed piezotube* is 200 times more efficient than in the plane standing wave resonator. This advantage can be increased a number of times if we use several resonance modes in the resonator, thus moving the particles from different nodes to the central node area [12].

Multifunctional piezotube

The last type of resonator is the *multifunctional piezotube*, which employs a combined mode of operation using both high-intensity, low-frequency (5 W/cm^2, 1 MHz) waves for separation and concentration of the large particles and low-intensity, high-frequency (0.5 W/cm^2, 20 MHz) waves for compositional analysis (Figure 4). Acoustic-radiation-force-induced motion of particles in the swept-frequency mode provided effective stirring and mixing of fluids in the *multifunctional piezotube* and resulted in high accuracy of sound velocity measurements (up to 0.0001%), and of absorption (about 0.5%), and rapid testing time (5-20s). Comparative analyses of the ultrasonic method with standard reference techniques have produced linear calibration curves for major components, with correlation coefficients higher than 0.95. It is thus possible to monitor directly in raw milk in cowsheds: protein and fat content and somatic cell count, or in drinking water salinity, turbidity, specific gravity, and bacteria.

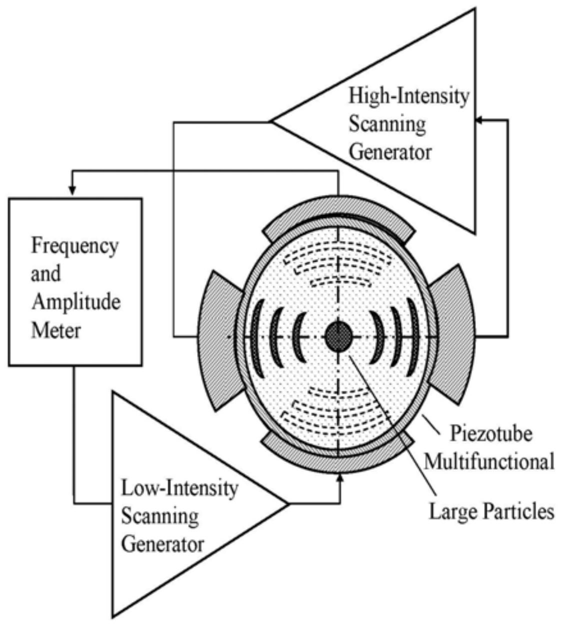

Figure 4. Ultrasonic device based on *multifunctional piezotube* combining high-intensity unit for separation of the particles and low-intensity unit for precise measurements of sound velocity and sound absorption

ANALYSIS BASED ON ACOUSTIC RADIATION FORCES

The new version of the cylindrical ultrasonic food analyzer is based on high-intensity standing waves for preliminary separation and concentration of the lardge particles (fat globules, somatic cells, yeasts) by the acoustic radiation forces and low-intensity standing waves to measure their content. Preliminary testing of the analyzer was carried out on the milk samples of five cows milked 2 times a day for one month. More than 300 samples of the raw milk from Kibbutz Tzora, Israel were tested and analyzed. Obtained results demonstrate the possibility of rapid measurements (20s) and a good correlation with the central certified laboratory for milk quality testing run by the Cattle Breeding Association of Israel. Continuous monitoring of milk fat and somatic cell count, which typically have high day-to-day variation, provide a much-needed tool for dairy management and for veterinary diagnostic purposes. It was found that milk production level, stage of lactation, and outside temperature have significant influence on the milk composition [11]. Ultrasonic technology can be incorporated as an add-on-line component in existing flow-through systems. This will enable continuous on-line monitoring of the quality of dairy milk, and will facilitate the prediction of mastitis by the determination of the main components: fat (accuracy (±0.04%), protein (±0.03%), sugar (±0.04%), somatic cells (±50,000 cells/ml or 30%).

LABELS FOR ULTRASONIC BIOSENSORS

Enzymes

Combining the acoustical manipulation of the particles and precise analysis in the *multifunctional piezotube* allows considerably improved ultrasonic monitoring of enzymatic and immune reactions. Sensitivity of acoustic detection of enzymatic reactions is determined by their rate and acoustic effect. In the case of low-molecular-weight substrates, the acoustic effect is mainly determined by changes in hydration. For high-molecular-weight substrates, one more effect influences a change in compressibility; the loss of a compact structure of biopolymers results in an increase of the acoustic effect by several orders of magnitude. This effect occurs to a great degree in the hydrolysis of starch and globular proteins (casein, ovalbumin), when intraglobular interactions are replaced by hydrational ones (Table 3). Calibration curves for all tested enzymes, and especially for hydrolytic enzymes, have extremely wide ranges of concentration measurements (four orders of magnitude) and high sensitivity (up to 0.4 ng/mL for α-amylase [13]).

Table 3. Acoustical detection of enzymatic reactions by precise measurement of sound velocity

Enzyme	Turnover number	Detection Limit ng/mL
α-Amylase	40,000	0.4
Urease	60,000	4
Protease, alkaline	2,000	5
Chymotrypsin	1,500	10
Creatine kinase	25,000	15

Bacillary α-amylase was proposed as an enhancer of the detected signals in the biosensors and as a label for ultrasonic enzyme immunoassay of different toxins and hormones in food products. This assay is based on activity modulation of an amylase-antigen conjugate as a result of conformational changes in the catalytic site caused by interaction with antibodies. The enzymatic activity is restored after adding the sample to be tested if it contains antigen [14].

Antibody-coated particles

The acoustical manipulation of the particles and high-frequency monitoring can considerably improve the speed and sensitivity of the widely used latex agglutination immunoassay of pathogens (*E. coli*, *Salmonella*). The latex immunoassay is based on agglutination of pathogens by antibody cross-linkages established when antibody-coated latex particles react with a pathogen. This assay is fast, but lacks sensitivity to small concentrations of pathogens. We used the *multifunctional piezotube* to accelerate the latex immunoassay and to provide quantitative determination of pathogen concentration by monitoring change in sound velocity. This is exemplified for Rota-virus, *E. coli* and *Salmonella* [15-17]. We used high ultrasonic intensity and a laminar flow to trap and concentrate selectively bacteria attached to antibody-coated latex particles. Under the conditions chosen [15], the acoustic radiation forces acting on attached bacteria drives them directly to the node (Figure 5). Free latex particles, as well as free bacteria, are not trapped and are washed out of the separation area by the aqueous flow, resulting in high specificity and sensitivity of the assay. Ultrasonic method enabled sensitivity down to $3{\cdot}10^5$ CFU/mL *E. coli* O157 and $8{\cdot}10^5$ CFU/mL *Salmonella*, compared to sensitivity of $1.7{\cdot}10^7$ CFU/mL *E. coli* O157 and $1.3{\cdot}10^8$ CFU/mL *Salmonella* obtained by the standard latex agglutination immunoassay. Since response time is critical, real-time monitoring is much more effective than physical sampling-based-monitoring with sample collection frequency of 24 hours.

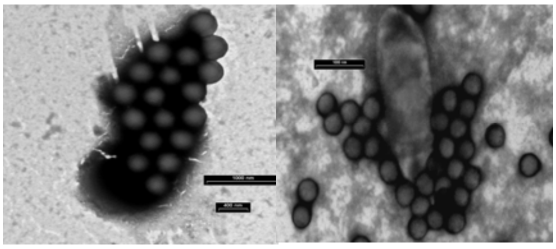

Figure 5. Electron micrographs of *E. coli* (left) and *Salmonella (*right*)* pretreated by ultrasonic standing waves

SUMMARY

The direct analysis of food products by cylindrical ultrasonic devices allows simultaneously determining 3-5 components at concentrations as low as 0.1% in 5-20s. Using cylindrical acoustic radiation forces allows one to increase the sensitivity of determining large particles (fat globules, bacteria or yeast) and to reach a detection limit as low as 0.0001%. Further increasing of the specificity and sensitivity of the ultrasonic analysis of biologically active compounds (hormones and toxins) in food products can be achieved by using enzymes or antibody-coated particles as labels.

The described liquid analyzers based on cylindrical ultrasonic standing waves are very effective for continuous monitoring of quality of various food products, such as milk, juices, wine, and drinking water. The cylindrical measurement chamber is optimal for on-line monitoring and easy cleaning. Advantages of the proposed technology include its reagent-free nature, no need for sample pretreatment, ease-of-use, and low cost.

ACKNOWLEDGMENTS

This study was supported in part by grants from the Office of the Chief Scientist of the Ministry of Industry and Trade of Israel and the Water Authority of Israel. We thank Prof. Armen Sarvazyan for initiating this research and for useful discussions.

REFERENCES

1 A. Priev and A.P. Sarvazyan “Cylindrical standing wave resonator for liquid food quality control" *J. Acoust. Soc. Am*. **125** (4): 2593 (2009)

2 A.P. Sarvazyan “Ultrasonic velocimetry of biological compounds” *Annu. Rev. Biophys. Biophys. Chem.* 20: 321-342 (1991)

3 R.E. Challis, M.J.W. Povey, M.L. Mather and A.K. Holmes “Ultrasound techniques for characterizing colloidal dispersions*” Rep. Prog. Phys.* **68**: 1541-1637 (2005)

4 Z.J. Dolatowski, J. Stadnik and D. Stasiak “Applications of ultrasound in food technology*” Acta Sci. Pol., Tecnol. Aliment.* **6**(3): 89-99 (2007)

5 M. Wiklund and H.M. Hertz “Ultrasonic enhancement of bead-based bioaffinity assay*” Lab. Chip* **6**(10): 1279-1292 (2006)

6 M. Hill and R.H. Nicholas “Ultrasonic particle manipulation*”* In: *Microfluidic technologies for miniaturized analysis systems.* Chapter 9 (2007)

7 A.P. Sarvazyan and V.P. Ponomarev “Method and apparatus for measuring acoustic parameters in liquids using cylindrical ultrasonic standing waves*” US Patent* 5,533,402 (1996)

8 A. Priev and O. Garbuzenko “Application of ultrasonics for quality control of drinks” In: *Ultrasonics International* (Delft, Netherlands, 2001) p. 110

9 A. Priev, V.P. Ponomarev and A.P. Sarvazyan “Method and apparatus for determining the composition of fluids” *US Patent* 6,920,399 (2005)

10 A. Priev, S. Zalipsky, R. Cohen and Y. Barenholz “Determination of critical micelle concentration of lipopolymers and other amphiphiles. Comparison of sound velocity and fluorescent measurements” *Langmuir* **18** (3): 612-617 (2002)

11 D. Priev, V.P. Ponomarev and A. Priev. “Acoustic radiation forces in monitoring of milk composition” *J. Acoust. Soc. Am.* **123** (5):3789 (2008)

12 A. Priev and V.P. Ponomarev “Method and apparatus for determination of the composition of particles in multi-component fluid systems” *US Patent* 7,484,414 (2009)

13 A. Priev, O.D. Hendrickson, O. Garbuzenko, A.V. Zherdev and B.B. Dzantiev “Development of a portable ultrasonic immunosensor and its application for pesticide detection” In: *Proceedings of the Eurosensors XVII*, Guimaraes, Portugal, pp. 242-244 (2003)

14 A.V. Zherdev, A. Priev, O.D. Hendrickson, A.A. Martianov, D.V. Sotnikov and B.B. Dzantiev “Monitoring of enzymatic and immunochemical reactions based on acoustic changes in solutions” *Phys. Pat. Immune System* **9** (7): 31-36 (2005)

15 D. Bavli, Y. Barenholz, N. Emanuel, V.P. Ponomarev and A. Priev “Ultrasound-enhanced latex immunoassay of pathogens in water” *J. Acoust. Soc. Am.* **123** (5):3789 (2008)

16 D. Bavli, Y. Barenholz and A. Priev “Ultrasound-enhanced nanoparticle-based monitoring of bacteria in water” In: *Proceedings of the Euronanoforum,* Prague, Czech Republic, P-072 (2009)

17 D. Bavli, Y. Barenholz and A. Priev “Ultrasonic quantification of nanoparticle-based immunoassay of Salmonella in water In: *Proceedings of the Euronanoforum,* Prague, Czech Republic, LP-13 (2009)