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Detection of microbial contamination in fruit juices using non-invasive ultrasound

Luis Elvira [a,*], Carmen M. Durán [a], José Urréjola [b], Francisco R. Montero de Espinosa [a]

[a] Centro de Acústica Aplicada y Evaluación no Destructiva, CSIC, Serrano 144, Madrid 28006, Spain
[b] B.C. Aplicaciones Analíticas SL, Josep Anselm Clavé 32-34, Esplugues de Llobregat 08905, Barcelona, Spain

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ABSTRACT

The aim of this work is to analyze the viability of using non-invasive ultrasonic methods to detect microbiological contamination in industrial fruit juices. Microbial growth in UHT fruit juices was monitored by an ultrasonic measuring system based on pulse transmission. The wave time of flight across different media was registered continuously during incubation. Three types of juices were used as substrate: apple juice, orange juice and peach&grape juice, and three different microorganisms were separately inoculated in these juices: bacteria (acidolactic mixture), yeasts (*Saccharomyces cerevisiae*) and molds (*Aspergillus niger*). It is shown that time of flight evolution is clearly affected by the growth of such microorganisms, these changes being dependent on the juice and the contaminant agent. These results support the viability of using ultrasonic measuring devices to assess the microbiological quality control in juices, decreasing detection times and reducing economical wastes when contaminations appear.

1. Introduction

Detecting microbiological contaminations during production is a matter of primary importance in the food industry. Although acidic features of some foods like fruit juices limit the number and variety of potential biological contaminators, different agents are able to survive and grow degrading the organoleptic and healthy characteristics of these products. Such degradation may have a remarkable negative commercial impact when these foods reach the consumers. In addition, some microorganisms fermentate juices with a high amount of gas production, which may lead to a violent breakdown and spoilage of juice packs during the storage at the factory and giving rise to significant environmental drawbacks and economical wastes.

UHT sterilization almost eliminates microbiological hazards in foods. Nevertheless, failures in sterilizers or conductions and contaminations during packaging may leave viable microorganisms in the product. To assess the final quality control of processed juices, different methods are used in the industry. Samples are separated from each production batch, incubated at a certain temperature (often around 30 °C) and analyzed. There is a wide variety of methods for carrying out these analysis, from the most traditional ones, like microscopy and plate counting (Wareing & Davenport, 2007), to rapid microbiological methods like ATP bioluminescence or CO_2 detection (Pishawikar, Singhal, & Kulkarnikl, 1992).

Non-invasive techniques based on ultrasound propagation have proved to be useful for the quality control in the food industry (Nollet, Toldrá, & Hui, 2007), as the measurements made by such techniques can be related to different food characteristics. It has been shown that microbiological growth gives rise to changes in the mechanical properties of the medium which can be detected using non-invasive ultrasonic methods. Such detection was achieved in microbiological culture media (Maestre & Montero de Espinosa, 2001; Sierra, Elvira, García, Resa, & Galán, 2009; Zips & Faust, 1989) and also in food products like soup and milk (Hæggström, 1997). Recently, Elvira et al. (2005) developed a multichannel device based in ultrasound able to detect microbiological contaminations in UHT milk carton-laminated packs without opening them. This device was used to perform a non-invasive monitoring of those milk packs taken out of the production chain for analysis during the incubation time. This operating procedure allowed a reduction of the time needed to detect microbiological contaminations. The aim of this work is to analyze the viability of using the same procedure to detect microbiological contaminations in fruit juices.

Although ultrasonic methods were applied to characterize different properties of fruit juices like sugar content (Contreras, Fairley, McClements, & Povey, 2007) or solid content and titratable acidity (Valente, Prades, & Laux, 2013), up to the authors' knowledge there is no study about ultrasonic monitoring of microbiological

* Corresponding author. Tel: +34915618806.
E-mail address: luis.elvira@csic.es (L. Elvira).

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contamination in fruit juices. To face the proposed first approach to this problem, a laboratory ultrasonic device (Elvira, Durán, Sierra, Resa, & Montero de Espinosa, 2007) was used to monitor continuously the ultrasonic time of flight in juice samples. Following Wareing and Davenport (2007), yeasts are the most significant group of microorganisms associated with spoilage of fruit juices, in addition some mold species and acid tolerant bacteria are also common contaminants of this product. Therefore, a yeast (*Saccharomyces cerevisiae*), a mold (*Aspergillus niger*) and a mixture of acidolactic bacteria were chosen for this study, and inoculated separately into three different juices extracted from apple, orange and peach&grape. These juices have different physical characteristics (density, viscosity and solid contents) and were selected as representatives of the wide variety of industrialized UHT juices.

Both sterile and inoculated samples were analyzed. Plate counting was made to check the results obtained using ultrasound, mainly to confirm or discard the growth of the inoculated microorganisms. The influence of the substrate and the contaminating agent was analyzed and a first approach to the range of detection times expected is presented. The results obtained show the viability of using this ultrasonic method to assess the microbiological quality control in fruit juice production.

2. Materials and methods

Three different types of commercial UHT juices were provided by the company AMC and used as substrate for microbiological growth detection:

- Apple juice: clarified juice.
- Orange juice: turbid juice with solid particles.
- Peach&grape juice: turbid juice with a high viscosity.

Physical-chemical properties of these juices are detailed in Table 1. To contaminate these juices, three different microorganisms were chosen among molds, yeasts and bacteria. The strains, selected by BC Aplicaciones Analíticas and AMC group are:

- *A. niger* in solid pellets WDCM 00144 (Microkit Laboratories). Solution of the pellet into 1ml of Ringer's solution (¼ strength from Fisher Scientific) gives 10^4 c.f.u./ml.
- *Acidolactic bacteria mixture* in solid pellets MKTB 0038 (Microkit Laboratories). Solution of the pellet into 1ml of Ringer's solution gives 10^5 c.f.u./ml.
- *S. cerevisiae* in solid pellets WDCM 00058 (Microkit Laboratories). Solution of the pellet into 1ml of Ringer's solution gives 10^4 c.f.u./ml.

Cultures in Petri plates from innoculi and juice samples were done using MRS Agar (Microkit Laboratories) for *S. cerevisiae* and acidolactic bacteria and Bengal Rose Chloramphenicol Agar (Microkit Laboratories) for *A. niger*.

2.1. Sample preparation

Four 125 ml glass bottles were autoclaved and 100 ml of sterile juice was added to each bottle. 1 ml of Ringer's solution was heated at 37 °C in an oven during 10 min. Then the pellet was added to this solution and stirred with a vortex until it was completely solved. Following the provider, the cell concentration obtained was 10^4 c.f.u./ml for *A. niger* and *S. cerevisiae* and 10^5 c.f.u./ml for the acidolactic bacteria mixture. Then, this solution was incubated in the oven at 37 °C during 20 min. After that, the solution was diluted in ringer again to obtain 10^3 c.f.u./ml, 10^2 c.f.u./ml and 10^1 c.f.u./ml respectively which was used to inoculate the juice. In addition, a sample from the 10^1 c.f.u./ml solution was spread over the surface of the corresponding agar in a Petri dish and incubated in the oven at 37 °C during 48 h.

Each juice bottle was inoculated with 1 ml of microorganism solution to start the experiment with 10, 1 and 0.1 c.f.u./ml respectively. 1 ml of sterile ringer was added to the forth juice bottle which was used as a sterile control. After inoculation, juices were placed in a water bath during half an hour at 30 °C to accelerate the thermal stabilization. Finally, they were placed into the Milisound equipment, which was also set at 30 °C for ultrasonic monitoring.

Table 1
Properties of the juices at 20 °C.

Juice	pH	Density (kg/m^3)	Dynamic viscosity (Pa s)
Apple	3,7	1044	0,020
Orange	3,6	1044	0,040
Peach&grape	3,8	1046	0,100

2.2. Ultrasonic monitoring

Ultrasonic measurements were made in the *Milisound* measurement system (Elvira et al., 2005). This equipment allows a four channel simultaneous measurement. It consists of four independent thermostatic chambers to keep a stable temperature in the samples for incubation and analysis.

The ultrasonic measurement is achieved by a through-transmission technique, one ultrasonic transducer is used as emitter and another different transducer is used as receiver in each channel. The ultrasonic wave passing through the sample carries information about the state of this sample. All the transducers are made of PZT 27 (Ferroperm) piezoceramics. Pulse excitation during analysis consisted of 20 cycle tone bursts centred at 4 MHz. The time of flight, which is the time needed for the wave to go from the emitter to the receiver, is monitored continuously during in a period between 2 and 6 days, depending on the case. The changes of this time of flight are related to the physical–chemical changes taking place in the juice as a result of the thermal equilibrium, particle settling or medium composition during microbiological growth. Once the analysis finished, a sample is taken from each bottle for microbiological counting.

2.3. Final microbiological counting

Samples from each bottle were used to make seriated dilutions down to seven concentration orders in Ringer's solution. In the case of the control bottle, no dilution of the sample was made. Agar plates are seeded and incubated at 37 °C during 48 h. After this time, plates containing 30–300 colonies were considered to determine the microorganism concentration of the samples.

3. Results and discussion

Having in mind the long duration of the experiments, in this preliminar stage of the ultrasonic monitoring of juice research only one run was performed by each microorganism and juice combination. Three main questions are discussed in this section: the general effect of microorganism growth on the ultrasonic time of flight in juices, the influence of some relevant variables (type of juice, initial concentration and nature of the microbial strain) upon growth detection and, finally, the time needed to achieve such detection.

3.1. Microorganism counts

Microbiological counting was performed, in first place, to confirm the viability and concentration of microorganisms at the