NON-INVASIVE IMPEDIMETRIC STERILITY TESTING OF ASEPTICALLY PACKAGED FOOD PRODUCTS

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Abstract

In the present article a brief overview of the most promising non-invasive sterility test methods is given, with their strengths and weaknesses. They are all inspired from medical technology and are a part of An International EUREKA Project, called "ENDTEST". The project started in December 1994 and involves research on a wide variety of non-invasive sterility tests.

Also, the initial results of application of the impedimetric approach to the problem are presented. The measurements have been carried out by placing conducting plates, acting as electrodes, on two opposite sides of the carton and measuring the impedance between them. Finally, comments on the preliminary experimental results and some conclusions are presented.

I. Introduction.

The goal of the aseptic packaging of food is to create a long lasting commercial sterile product. The definition of such a product, as given by the American Food and Drug Administration, is: "The absence of micro-organisms, capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution".

Processing and packaging sterile food-stuffs is a complicated production process, involving preliminary sterilisation of the product and the packaging material, ensuring sterile environment during packaging and, of course, creating tight and steady food containers as an output product. On-line inspections and quality controls during the production process are, by all means, useful, but checking the tight container and its product content before it enters the distribution chain, is the quality control that today is considered the most important one, as it covers all the preceding procedures.

If we look for an end-test of the whole production lot, we have no other alternative, but to use non-destructive methods. There exist three basic criteria for evaluating the applicability of different methods: *nonspecificity* in order to ensure detection of all types of bacteria; *sensitivity* in order to minimize the necessary incubation time; *rapidity* in order to permit extensive testing. [1]

We shall mention here three promising new methods, that are inspired from medical technology, and that are under development within the International Eureka "ENDTEST" project.

1. *Ultrasound Imaging Method.*

This method was originally developed at the Technical Research Centre of Finland and is considered to be the first serious attempt, explicitly aimed at developing a new non-invasive sterility test. The method is based on the change of ultra sound images, because of structural changes of the packaged food and gas forming, caused by microbial enzymes. The image is created by scattering of 5MHz acoustic waves in the tissue. Its advantages are rapidity and sensitivity, that makes it suitable for testing large samples after only 3-7 days of incubation. However, in order to be commercially viable, it is necessary to be able to detect spoilage through cardboard. One disadvantage of ultrasound imaging is that at frequency of 5MHz the penetration of ultrasound impulses into the package is restricted by the big specific acoustic impedance of the cardboard. At lower frequencies (0.5-2 MHz) this problem is overcome, but then only larger particles of the food product reflect the ultrasound and in this way the sensitivity drops down. At higher frequencies (7.5 MHz) the sensitivity goes up and normally existing fat particles in the food are detected as well. This gives a slightly turbid image of sterile food, as well, and may lead to confusion. In order to make ultrasound images more useful and practical for routine assessments they can be digitalized. Images in the numerical form are easier to interpret than the original ones. [1]

2. *Ultrasound Doppler Method.*

This is another non-invasive ultrasound based method, proposed by H. Gestrelius *et al.* - Sweden [2]. An ultrasonic beam, transmitted into a liquid, creates a controlled motion, called ultrasonic streaming. The velocity of this streaming depends on physical parameters, such as viscosity, sound absorption, speed of sound and probably other factors, that might be affected by microbial activities within the food. The velocity spectrum of the ultrasonic streaming is assessed by measuring the Doppler shift of the reflected sound waves from the particles or gas bubbles in the food. The faster the particles move away from the ultrasound transducer, the greater is the Doppler shift.

The potential of this method lies in the fact that a measurement can be done within 10 seconds, and that it seems to be sensitive enough to detect unsterile packages after only a few days of incubation. The fact that the method is rapid opens up the possibility of 100% testing of the production lot, while the high sensitivity minimizes the necessary incubation time, before a product can be sold on the market. Limiting factors include that it only works on liquid food-stuffs, and the possibility some microbial growth, that induces very weak textural changes of the product, not to be detected. [2]

3. *Calorimetric Method.*

Metabolic active and growing microorganisms consume energy, part of which is used to generate a certain amount of heat. G. Meijer *et al.* [3] has developed a concept in which the small temperature increase of the product, caused by the growing microorganisms, can be detected with highly-sensitive smart temperature sensors. By means of thermal insulation of the packages from the surrounding environment, the heat dissipation is considerably decreased, which gives a temperature rise of the inoculated packages up to 0.7°C. The great potential of this method lies in the fact that it, by measuring energy dissipation, which is directly coupled to all organic growth, actually succeeds in combining high sensitivity with a low specificity. A limitation is the fact that measurements have to be done before and during the actual microbial growth. Since the exact timing of this is uncertain, each package must be assessed over a 10-20 hours time span under special conditions (thermal isolation). Hence, the method is more suitable for testing smaller batches and selective testing of larger batches.[3]

Unfortunately, at this moment we are not aware of a method, including the above mentioned, that meets fully the three basic criteria. This is a big challenge to research workers for further improvement of the already existing methods or developing new ones.

II. Impedance Method.

Nowadays it is well known that microbial metabolism usually causes an increase in both conductance and capacitance of the nutritious media and therefore, as micro-organisms grow, the impedance of the media is tending to decrease. The usual implementation of this method requires direct contact between the measuring electrodes and the media under control, which is not possible in the case of sterility control of aseptically packaged foods, because of the non-invasivity requirement.[4] Therefore, instead of measuring directly the impedance of the food product, we have to do it in indirect way by measuring the impedance of the whole package.

The measurements were carried out by placing conducting plates, acting as electrodes, on two opposite sides of the carton (Fig.1) and measuring the impedance between them.

One of the problems under investigation was to establish if a change of the impedance of the packaged food gives a distinguishable change of the total impedance of the package, which includes also the impedance of the packaging material. It appeared that the impact, if any, of the food impedance on the total impedance is very small. This is due to one of the inner layers of the packaging material, which is an aluminium foil, acting as a Faraday's cage and not allowing the electric field lines to pass through it.

The initial experimental results, utilizing the HP4194A Impedance/Gain-Phase Analyzer, showed that the measured impedance has a well defined capacitive character in frequency range 100Hz ÷ 20MHz, as the value of the phase angle is very close to -90 deg

(within -87deg ÷ -89deg). So, from now on we shall speak about capacitance measurements rather than impedance.

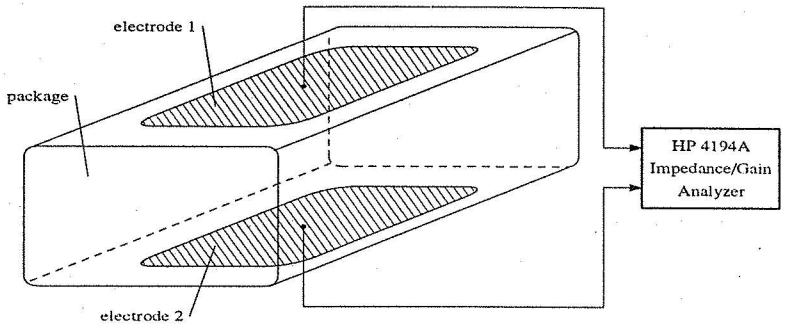

Fig.1 Capacitive method for sterility control of aseptically packaged foods.

The equivalent capacitance C_Σ is formed by two capacitors in series, as it is shown in Fig.2.

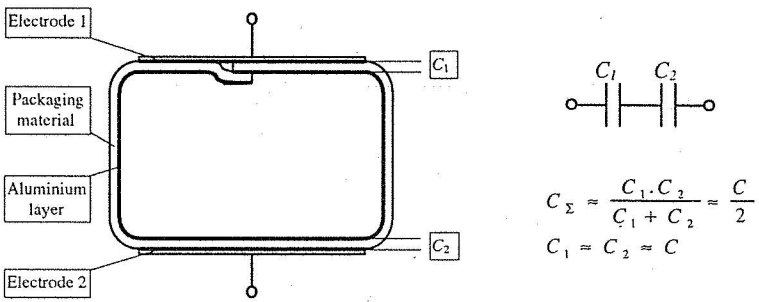

Fig.2 A cross-section of a package with two electrodes on opposite sides and an equivalent electric circuit.

At this moment we cannot say with certainty if this method is sensitive to impedance changes of the packaged food, due to bacterial metabolism, or not. The reason is that the method appeared to be very sensitive to volume changes of the carton itself, due to gas production of some kind of bacteria or temperature alterations. The temperature

change of the package could result from environmental temperature alterations or from heat, produced within the package.

This unexpected result gave birth to another idea: to use the impedimetric approach for monitoring what's going on with the package from outside rather, than to try to pass signals through it. To examine the package outwardly is not a new idea, because measuring the temperature of the outer surface of the packaging material is following the same principle. The new element in this idea is that yet another physical phenomenon could be used to detect bacterial growth - the change of the volume of the carton. This change, if due to gas production, is irreversible and do not require constant monitoring in order to be registered. So the method may be considered *rapid* and *sensitive* one, if used to detect gas producing bacteria. Unfortunately not all the bacteria possess this property. If used to detect heat production in the package, it gains all advantages of the calorimetric method, being even more *non-specific*, as it will detect volume changes of different origin.

III. Experimental results.

Some experiments were carried out utilizing this already pseudo-impedimetric "sandwich method" (UHT-milk carton placed between two conducting plates with fixed distance between them). One UHT-milk carton was inoculated with gas producing bacteria *E.coli* and another was sterile. The results showed that after 24 hours the initial capacitance of the inoculated package changed with 50% and after another 24 hours the capacitance change was 300% (Fig.3). The capacitance of the sterile package was

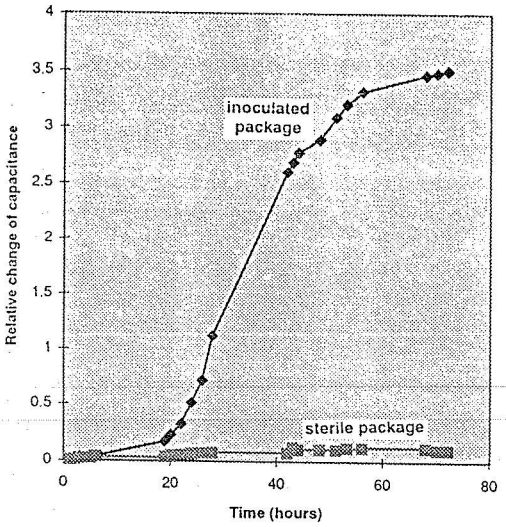

Fig.3 Relative change of capacitance of inoculated with gas producing bacteria milk package and a sterile milk package, versus time.

monitored for longer period of time (10 days) and it changed within 10%, mostly due to variation of the room temperature. Also, we were interested in what is the tolerance of the capacitance of different sterile packages of one and the same kind. Measuring the capacitance of 12 sterile UHT-milk packages gave a tolerance of 25%, while a repeated measurement of the capacitance of one package, with constant distance between the electrodes, gave a tolerance of only 5%.

The next experiments were intended for evaluating the sensitivity of the method to temperature changes of the package. The temperature was measured in the same way it is measured in the *calorimeter*, described in [3]. The package was sterile and its temperature variations were due to variations of the room temperature. In this way we were sure that during the experiment there will be no other reasons for changing the volume of the package but temperature. Measurements were carried out for more than 10 days. In Fig.4 is depicted the relative change of temperature T and the capacitance C of the package versus time and in Fig.5 is presented the dependence of the relative change of the capacitance C to the relative change of temperature T of the package with polynomial approximation of 2nd order.

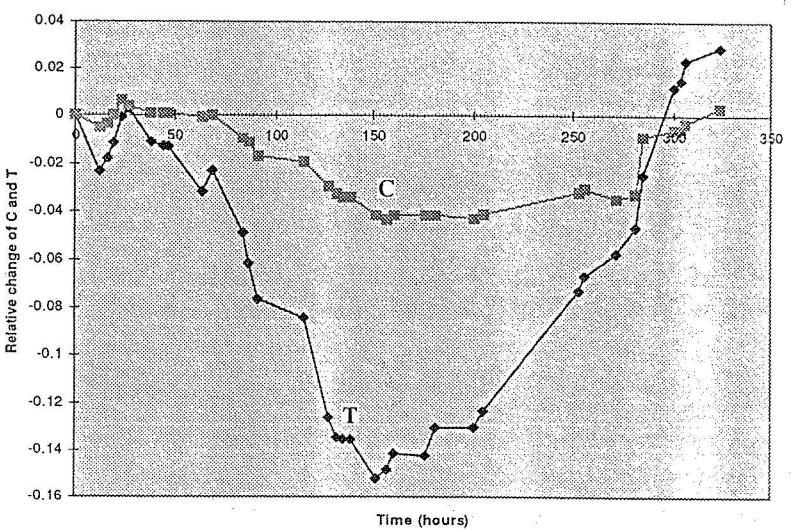

Fig. 4 Relative change of temperature T and capacitance C versus time

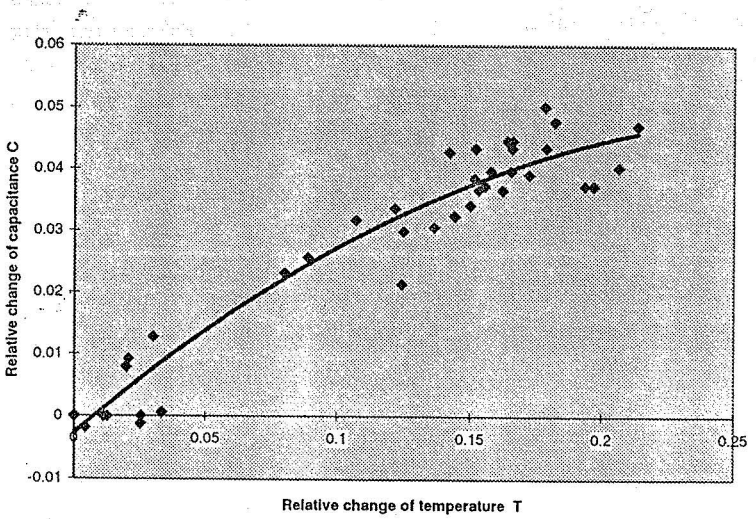

Fig. 5 Dependance of the capacitance Cs on the temperature of the package
Polynomial approximation of 2nd order

IV. Conclusions.

The main conclusions we can draw from the experimental results obtained, are:

The impedance method, in the way used in microbiology, is not applicable for non-invasive sterility control of aseptically packaged foods, because of the properties of the packaging material and especially because of the presence of a conducting aluminium layer.

By measuring the capacitance of the package, fixed between two conducting plates, small changes of its volume can be detected. The volume change is used as an indicator of metabolic activities, either because of the heat production, or because of gas production of certain types of bacteria.

The method can be considered rapid and sensitive one, if used to detect gas producing bacteria, as less than 24 hours are needed for obtaining reliable result. If used to detect heat production in the package, the pseudo-impedimetric approach is an alternative of the calorimetric method and can pretend for universality, equal to that of the calorimetric method.

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