INVESTIGATION OF ANTIMICROBIAL INHIBITION EFFECT OF QUINCE FRUIT EXTRACT BY RAPID IMPEDANCE METHOD

Gabor Zsivanovits[1], Fruzsina Szigeti[2], Csilla Mohacsi-Farkas[2]
[1]Food Research and Development Institute, Plovdiv, Agricultural Academy, Bulgaria;
e-mail: g.zsivanovits@canri.org
[2]Corvinus University of Budapest, Dept. of Microbiology and Biotechnology, Hungary

Abstract

The aim of the present work was to examine antimicrobial effect of extracts from quince fruits for foodborne pathogenic bacterial strains. This effect was investigated by rapid impedance method (RABIT, Don Whitley Scientific Ltd.). In comparison of impedance microbiology with the traditional plate counts on culture medium method, it is much faster and automatized. During the tests for 24 hours, the samples were inoculated with a suspension of bacterial strains about 10^4-10^5 cell number (CFU/g) for observing the inhibition effect. For quantitative analysis of the results a calibration has been done on a dilution series. Based on our results the effect of extracts could be shown by decreasing of the integrated area of the impedimetric growth curve.

Keywords: antimicrobial effect of natural material, foodborne pathogenic bacterial strains, calibration of rapid impedance microbiological method,

ИЗСЛЕДВАНЕ ЕФЕКТА НА АНТИМИКРОБИАЛНО ИНХИБИРАНЕ НА ЕКСТРАКТ ОТ ДЮЛЯ С БЪРЗ ИМПЕДАНСЕН МЕТОД

Габор Живанович[1], Фружина Сигети[2], Чила Мохачи-Фаркаш[2]
[1]Институт на Изследване и Развитие на Храни, Пловдив, Селско-Стопанство Академия, България; e-mail: g.zsivanovits@canri.org
[2]Корвинус Университет, Будапеща, Катедра на Микробиология и Биотехнология, Унгария

Резюме

Целта на настоящата работа е да се изследва антимикробиалния ефект на екстракти от дюля (цял плод) върху хранителни патогенни бактериални щамове. Този ефект беше изследван с бърз импедансен метод (RABIT, Don Whitley Scientific Ltd.). В сравнение с традиционния метод „директно изброяване на колониите след посявка и култивиране в агарови среди“, импедансната микробиология е много по-бърза и автоматизирана. По време на 24 часовите изпитания за наблюдение ефекта на инхибирането, пробите се инокулират със суспензия от бактериални щамове около 10^4-10^5 броя клетки (CFU/g). За количествен анализ на резултатите беше направено калибриране със серия на разреждане. Според нашите резултати ефектът на екстрактите може да бъде показан чрез намаляване на интегрираното пространство на кривата на импедиметрично нарастване.

Ключови думи: антимикробиален ефект на натурален материал, хранителни патогенни бактериални щамове, калибриране на бърз импедансен микробиологичен метод

1. Introduction

Fruits and vegetables contain essential oils such as eugenol (clove), allicin (garlic), cinnamic aldehyde and eugenol (cinnamon), allyl isothiocynate (mustard), eugenol and thymol (sage), thymol and isothymol (oregano) that have antimicrobial activity [10]. Naturally occurring substances in food work against the microbes, thus maintaining stability of food and these are directed toward a specific group of microorganism and have weak activity [11]. The inhibition effect depends on intrinsic factors (pH, water activity, redox potential/redox poising capacity), on the available

nutrients (sugars, water, minerals, vitamins and other growth-promoting factors), extrinsic factors (temperature, relative humidity, gas composition) of the environment and on implicit factors (general interferences of all other factors) of inhibitory substances [7].

The extract of quince peel and pulp has been recently investigated for antimicrobial effects. Their inhibition activity against different microorganism strains was also investigated. Quince peel was the most active for inhibiting bacteria growth with minimum inhibitory and bactericide concentrations. It seems that chlorogenic acid acts in synergism with other components of the extracts to exhibit their total antimicrobial activities [6].

Growing of bacterial strains occur changes in the bulk electrolyte solution (culture medium) due to metabolism of uncharged or weakly charged substrates which are converted to highly charged end products, e.g. proteins to amino acids. These changes can be monitored by impedance measuring. One of this type technique is the Rapid Automated Bacterial Impedance Technique (RABIT) (Don Whitley Scientific Ud.) [1-4]. In comparison of impedance microbiology with the traditional plate count on culture medium method, it is much faster and automatized. The impedance changes are typically measured by the use of a pair of electrodes placed within a growth medium or reacting solution (Figure 1) [9].

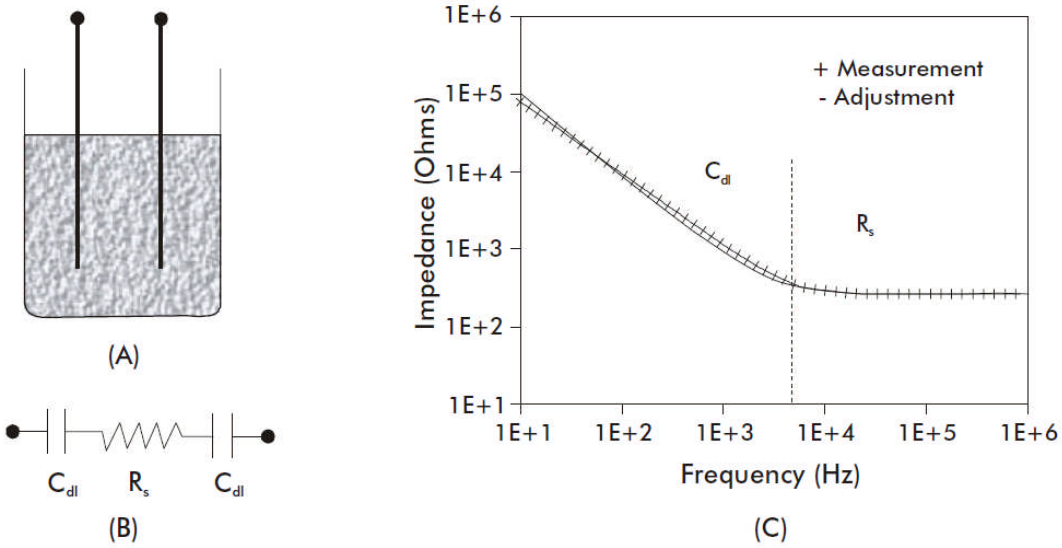


Figure 1. (A) Two electrodes method for impedance measurement; (B) Simplified circuit model; (C) Impedance curve vs. frequency [9]

In practical terms, the electrical signal is frequency dependent, has a conductive and capacitive component and is temperature dependent. The importance of temperature control in any impedance system is critical, as a temperature increase of 1°C will result in an average increase of 0.9% in capacitance and 1.8% in conductance [5]. The total impedance of the system depends on the impedance of the medium, impedance of the electrode and on the measure frequency (Figure 1C). The received curve can be properly interpreted by means of an equivalent circuit of the system. That simple circuit connected in series as shown in figure 1B, formed by the resistance of the solution between the electrodes (*Rs*) and the capacitors of the metal-sample interface (one for each electrode: *Cdl*) [9]. The impedance always decreases when the concentration of bacteria grows in the culture medium. The decrease in impedance has two causes: the decrease of Rs, and the increase of Cdl. It is acknowledged that bacteria metabolize uncharged large molecules producing small charged molecules, thereby decreasing the resistance of the medium (Rs) [9].

The impedance changes, which are the result of the growing of bacterial strains, can be detected in two ways. The first way is the direct conductometry, which is achieved by monitoring the charge changes (Proteins → Amino Acids, Carbohydrate → Lactate, Lipids → Acetatein) of the growth medium (Figure 2) [9]. The second way is the indirect conductometry, which monitors

changes due to evolution O_2 CO_2 produced by the metabolism of substrates in the culture medium (such as yeast and moulds) [1, 9]. The direct is applicable if the admittance of medium is increasing during the growing of bacterium. The indirect method measure the decreasing of medium admittance due to the produced O_2 CO_2. A calibration curve is produced for each organism, where the time to detection is directly proportional to the growth rate and indicative of real-time microbial activity.

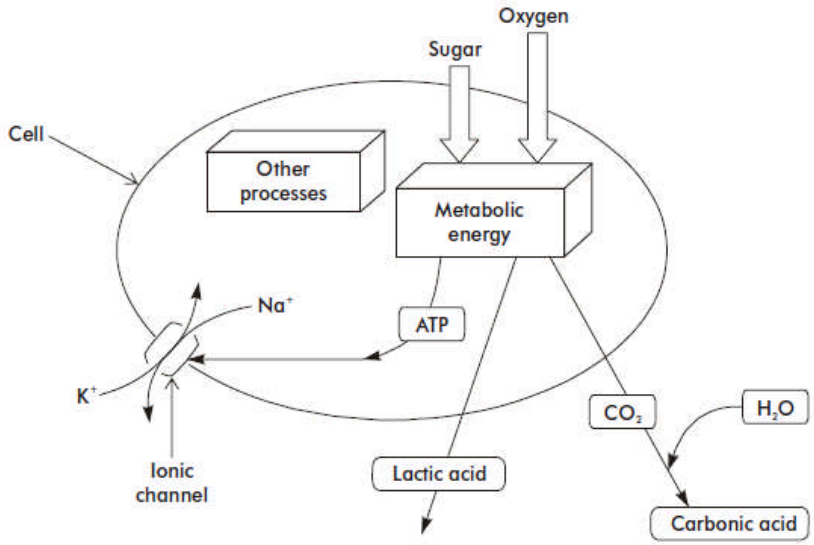


Figure 2 Direct conductometry (the metabolism of cells cause the charge changes). [9]

The Detection time means the time after which three successive increases in conductivity equal to or greater than a pre-determined value (Figure 3a) [9, 3] and depend on the number of bacteria cells (Figure 3b). The instrument has to be equipped with thermostat for constant and exact temperature and humidity control, because the growing of bacterium primarily depends on these parameters.

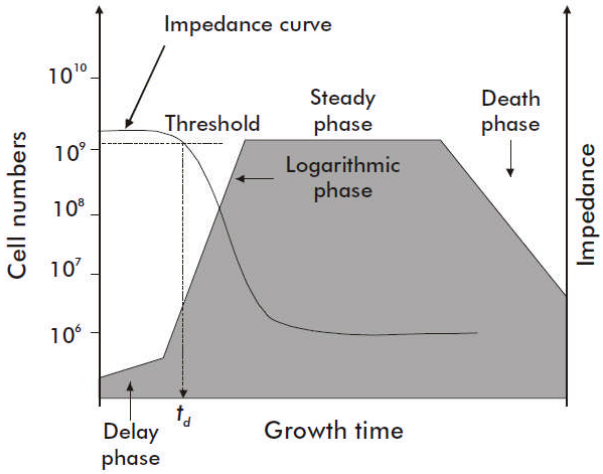


Figure 3a: Typical impedance curve of direct method with the detection time (t_d) [9]

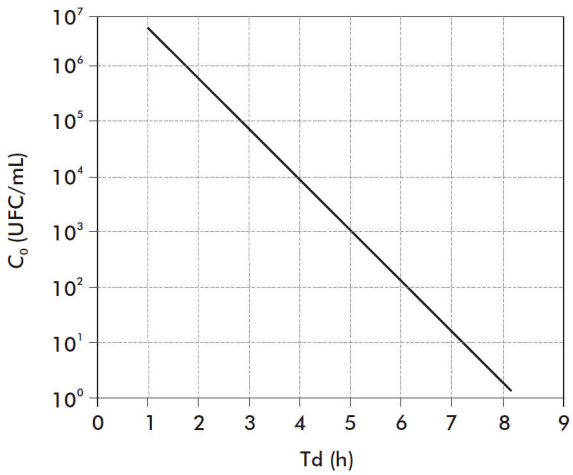


Figure 3b: Dependency between the detection time and the number of bacteria cells [9]

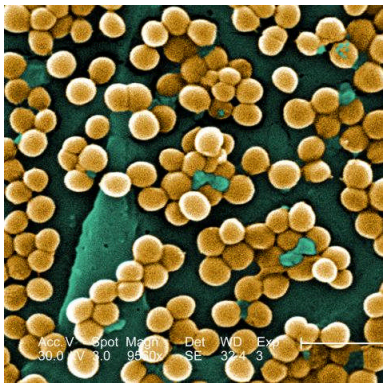
Quality-assured results are available more quickly than with traditional microbiological methods, improving both sample throughput and laboratory efficiency. Instead of inoculating large numbers of culture plates with different incubation times and temperatures, and is much less labour intensive and less time consuming. A versatile and cost effective rapid bacterial detection method, the RABIT is used to measure changes in the culture medium's electrical conductance that occur

when charged metabolites are produced by the bacteria of interest [2]. The method is capable for Coliforms, E. coli, Salmonella spp, Anaerobes, Gram negatives, Pseudomonas spp, Staphylococcus sp, Sporeformers, Yeasts & Moulds [4] and may be used in both quantitative and qualitative applications to detect all microorganisms or specific pathogens [12]. The method has potential for use to measure antimicrobial effect as well [2]. The shape of the growth curve can be a useful indication of the growth characteristics of organisms or groups of organisms, particularly if certain components in the growth medium are limited [3]. Typically, most impedance analysis of food samples can be completed in 6-24 h. This technique is not suited for testing samples with low number of microorganisms and that the food matrix may interfere with the analysis [8].

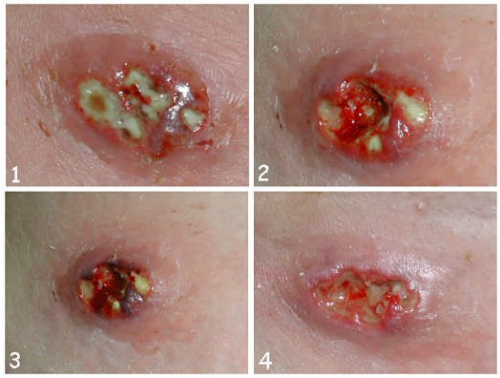
2. Materials and methods

For that study extracts of quince fruits (Cydona oblonga M.) variety Konstantinapolyi, (Figure 4) were used, infected by fresh culture of foodborn pathogenic bacterial strains (Staphylococcus aureus – Figure 5). The full fruits were used for extraction.

Variety: Konstantinapolyi
Figure 4 Fruit of quince



Bacteria
Figure 5 Staphylococcus aureus



Infections facts

Sample preparation

For the investigation of the antimicrobial inhibition effect the extracts from quince were inoculated with a suspension (CFU/g: 10^4-10^5) from fresh cultures in dilution series.

Experimental Method

The inhibition effect of probes was detected by rapid impedance method (RABIT, Don Whitley Scientific Ltd. – Figure 6) during tests for 24 hours.

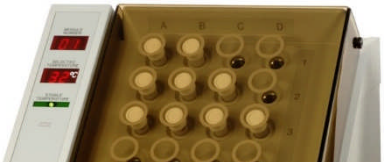


Figure 6 Instrumentation of RABIT method [4]

The method measure the admittance of solutions in μS. Exponential rising of admittance on the growth curves shows the time to detection (TTD). For calibration of the instrument dilution series and blind probes were used. Don Whitey (DW) impedance broth was used like culture medium at acidic pH 3 (adjusted by citric acid). The instrument measures the admittance of probes in every 6 minutes and draws an admittance curve.

The tubes of the instrument were filled by:

1. DW (blind);
2. DW + quince extract (to control the infection of the samples);
3. DW + suspense of pure bacterium;
4. DW + suspense of pure bacterium + citric acid (adjusted to pH3 to control the effect of acidic pH of quince extract);
5. DW + suspense of pure bacterium + quince extract.

Three repetitions were applied because in some cases the probes do not give interpretable curves. The information of antimicrobial inhibition effect is quantification from the area bellow long-drawn admittance curves.

3. Results and discussion

The Figure 7 shows the admittance in function of time for extract of Konstantinapolyi infected by Staphylococcus aureus. The green curves mean the growth of pure Staphylococcus aureus bacterium on DW culture medium; the blue curves show the admittance changes of Staphylococcus aureus bacterium on DW culture medium with citric acid; and the red curves present Staphylococcus aureus bacterium on DW culture medium with the extract of Konstantinapolyi.

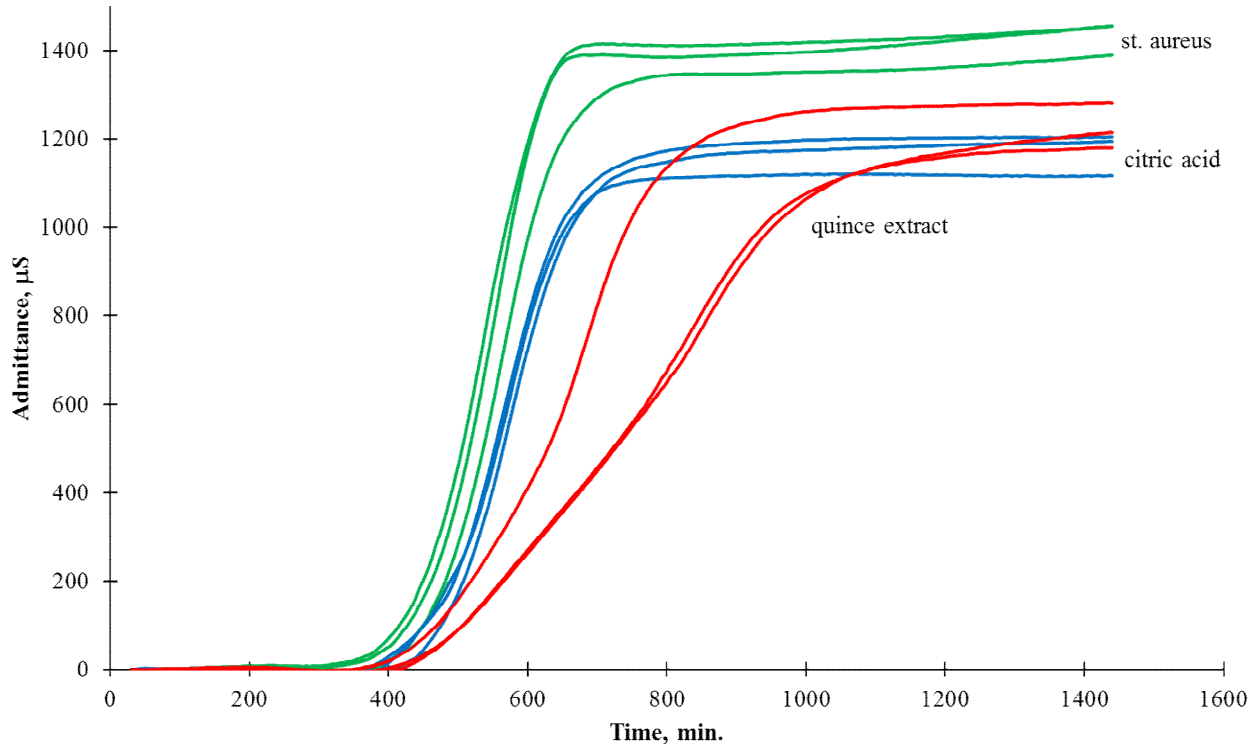


Figure 7 The admittance in the function of time for extract of Konstantinapolyi infected by Staphylococcus aureus

The detection time shows just small differences for infected samples, infected samples with pH 3 adjusted by citric acid and infected samples with extract of Konstantinapolyi quince variety samples – (368.0 ± 30.8) min, (406.0 ± 18.3) min, (422.0 ± 22.7) min respectively. The inhibition effect of quince extract is observed from the decreasing of the area below growth curve. The integrated areas are 1 256 014 ± 56 474.4 (100 ± 4.5%), 1 005 672 ± 32 851.3 (80 ± 2.6%) and 800 389.5 ± 137.9 (63.7%) respectively. The variance analysis shows the differences between the samples (Table 1).

Table 1 Variance analysis of the detection time and integrated area below growth curve

Sample name	*Count*	*Mean*	*St. deviation*	*Homogeneous Groups*		
		Detection time, min.				
St. aureus	3	368.0	±30.8	X		
Citric acid	3	406.0	±18.3	X	X	
Quince extract	3	422.0	±22.7		X	
		Integrated area				
Quince extract	2	800 389.5	±137.9	X		
Citric acid	3	1 005 672.0	±32 851.3		X	
St. aureus	3	1 256 014.0	±56 474.4			X

4. Conclusions

The quince's extracts have antimicrobial effect for foodborne pathogenic bacterial strains. The inhibition effect is detectable not in the extension of time to detection, but in the decrease of the integrated area bellow the impedimetric growth curve which is statistically different for the quince extract from the others. It means the rapid method is capable to show the inhibition effect of quince's extract on Staphylococcus aureus.

5. Acknowledgement

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