Estimation of microbial population of bitter chocolate mix by impedance measurement

Keimzahlbestimmung in bitter Schokolade Mischung mit Impedanzmessung

A. M. BATRINOU, E. D. KATSOGIANNOS, E. N. KOUSTOUMPARDIS, V. K. SPILIOTIS*

Summary:

This study evaluates the reliability of the impedance measurement for the estimation of microbial population of a specific type of chocolate used as raw material in the confectionery industry (bitter chocolate mix). A calibration curve by using artificially contaminated samples was elaborated for total aerobic count (log_{10} N) and impedance detection time (IDT in hrs), with threshold M-value at 5 %, demonstrating a good correlation between the two methods (R^2=0.89). Therefore, impedance measurement which is a more rapid, automated and less laborious method than aerobic plate count, could be used as an alternative method for the rapid detection of microbial levels in chocolate mix.

Key words:
Impedance measurement; microbial population; chocolate; confectionery

Zusammenfassung:

In der vorliegenden Arbeit wird der Einsatz der Impedanzmessung zur Keimzahlbestimmung in Schokolade untersucht (Bitterschokolade Mischung). Es handelt sich dabei um einen bestimmten Typ von Schokolade, der für die Herstellung von Konfekt verwendet wird. Für die Auswertung wird jene Inkubationszeit herangezogen, bei der ein Grenzwert der Impedanz (M-Wert 5 %) überschritten wird. Die „Impedance detection time" (IDT) wurde mit der Gesamtkeimzahl (Plattenverfahren) kalibriert, wobei die Korrelation mit R^2=0.89 sehr gut war. Für die Erstellung der Kalibrationskurve wurden experimentell kontaminierte Proben herangezogen. Im Vergleich zur standardmäßig angewandten kulturellen Keimzahlbestimmung über ein Plattenverfahren zeigt die Verwendung der Impedanzmessung die Vorteile, dass sie einerseits schneller und einfacher durchzuführen ist und andererseits durch die Möglichkeit zur Automatisierung die Reproduzierbarkeit erhöht werden kann. Es konnte in dieser Arbeit gezeigt werden, dass die Impedanzmessung für die Qualitätskontrolle von Schokolade, die als Rohmaterial für die weitere Verarbeitung dient, verwendet werden kann.

Kennwörter:
Impedanzmessung; Keimzahlbestimmung; Schokolade; Konfekt

Introduction

In recent years, emphasis is given in developing more rapid and automated methods in food microbiology. Automated methods are used to estimate the number, types and metabolites of microorganisms with various applications in the food industry (monitoring food spoilage, food preservation, food fermentation, food safety and foodborne pathogens). Indeed, rapid methods in microbiology offer the advantage of being less time consuming and less laborious and of requiring smaller volumes of media and reagents than

the conventional methods used in food microbiology (1). Impedance measurement is based on the principle that during microbial growth, metabolic processes produce electrically measurable changes in the growth medium due to the metabolism of high-molecular weight nutrients into smaller charged ionic components that increase the electrical conductivity of the medium. Variation in electrical conductivity is proportional to the change in the number of microorganisms and therefore microbial growth can be measured. Impedance measurement has been used for quality control in food industries (2) and especially in identification (3, 4, 5), enumeration (6, 7, 8, 9, 10, 11) of indicator microorganisms and of estimation of antimicrobial activity (12, 13). In impedance measurement the detection time (the time at which impedance change reaches a particular threshold) is recorded. "Impedance Detection Time" (IDT) is usually inversely proportional to the log number of microorganisms in the sample and therefore, microbial counts can be calculated or estimated by measurement of the IDT. Initially, a calibration curve must be adjusted to establish the correlation between total microbial population ($\log_{10}$ N) of a specific type of food with IDT. The calibration curve is designed by measuring the IDT of an adequate number of samples of known microbial population, determined by a conventional microbiological method such as the cultural aerobic plate count (APC). The impedance method has not been extensively applied in the food industry, mainly due to the fact that an efficient correlation must be first determined for every type of food.

The aim of this study was to investigate the reliability of the Impedance measurement for the estimation of microbial population of a specific type of chocolate used as raw material in the confectionery industry. Impedance measurement using the BacTrac™ 4000 system could be used as a rapid alternative method to screen bitter chocolate mix during food processing for the determination of acceptable levels of microorganisms. Bitter chocolate mix is one of the principal raw materials used for the manufacturing of chocolate products. To our knowledge, there are no studies in the bibliography investigating the use of the impedance measurement method in chocolate products.

Experimental Part

Materials and Methods

Sample preparation: Samples consisted of bitter chocolate mix (sugar 50 %, cocoa mass 46 %, cocoa butter 2.4 %, emulsifier (Crill 36) 1 %, flavouring (vanillin) 0.1 %, lecithin 0.5 %). This chocolate mix is used as finished product and also as raw material for the production of various confectionery products. Samples of 10 g (± 0.1 g) of chocolate were weighted in aseptic conditions, placed in polyethylene bags and diluted 1:10 with sterile peptone water. The mix was homogenized in a Stomacher (Colworth) for 5 min. For the estimation of the microbial population of samples two different methods were used, the Aerobic Plate Count (APC) and the impedance measurement method.

Aerobic Plate Count Method: Plate Count Agar (PCA; LAB M™) was used (prepared by mixing 23.5 g of PCA in 1 l of boiling distilled water, then sterilizing for 15 min in 121°C). After the preparation of the appropriate decimal dilutions of the sample, 1 ml from each dilution was transferred to sterile petri plates (in duplicate) and sterile, melted and cooled (44-46°C) agar was added. Petri plates were incubated at 29-31°C for 48 ± 3 hrs and colonies were counted.

Impedance measurement: The BacTrac™ 4000 system (SyLab, Purkersdorf, Austria) was used which consists of an incubator unit, which allows the simultaneous incubation of 20 test tubes of 10ml content each. Substrate used was the BiMedia 001A (for bacteria) sterilized at 121°C for 15 min. By means of an electrode system, mounted in the system's glass tubes (cells), impedance values can be registered over time during a pre-selected incubation period. Each incubator contains a microprocessor controlled electric unit for measurement and temperature control (4°C to 65°C). This system has the ability to evaluate simultaneously changes of the growth media impedance (M-value) and changes of the electrode impedance (E-value). In this study, measurable results were obtained by the M-value only. The level of the selected threshold M-value was 5 % (represents the decrease of the initial value of impedance) and the time needed for the M-value of each sample to reach that threshold is called Impedance Detection Time (IDT). The same samples used in the APC Method, in duplicate, were transferred in the system's glass tubes (1 ml from each dilution inoculated in 9 ml of substrate). The system was set to operate at 30°C for 24 hrs. After the incubation period was completed, a printout of the impedance patterns and the IDT expressed in hours for each test cell was produced.

Calibration curve: Samples with varying levels of microbial population ranging from 10^0 to 10^9/g were analyzed. In order to obtain an enriched culture of the endogenous flora of chocolate, a sample of the initial chocolate mix was diluted 1:10, homogenized and incubated at 30°C for 24 hours with continuous stirring. Serial decimal dilutions of the enriched culture were inoculated (0.1 ml) into diluted 1:10 and homogenised initial chocolate mix, generating samples with varying

microbial concentrations. Samples were analysed in duplicate by both the APC Method and the impedance measurement method using the BacTrac™ 4000. For each sample, a pair of data is obtained: the microbial population ($\log_{10}$ N) and the IDT (in hrs). A total of 27 data pairs were subjected to linear regression analysis to adjust a regression line.

Results

The decimal logarithms of microbial number (N) estimated by the APC method and the corresponding impedance detection time (IDT) determined by the BacTrac™ 4000 system (expressed in hours) are shown in *Table 1*. A linear regression model was adjusted such as y=*a*+*b*x where y = $\log_{10}$ N, x = impedance detection time in hours, *a*=intercept, *b*=slope. The regression model is represented mathematically by the equation:

$$\log N = 8.8219 - 0.8545 \cdot IDT$$

The regression line is shown graphically in *Figure 1*. Also, the coefficient of determination (R^2) and the standard error of estimation of the predicted value (s_e that mea-sures the reliability of the estimating equation) were calculated (*Table 2*).

no.of sample	logN	IDT (hrs)
1	0,52	11,2
2	0,65	9,16
3	1,08	8,43
4	1,65	8,95
5	2,0	7,07
6	2,43	7,79
7	2,98	5,86
8	3,51	6,16
9	3,7	4,36
10	3,95	5,83
11	4,0	4,8
12	4,78	4,12
13	5,04	3,66

no.of sample	logN	IDT (hrs)
14	5,04	4,96
15	5,15	3,59
16	5,23	4,42
17	5,3	4,32
18	5,36	4,3
19	5,45	4,26
20	5,46	4,21
21	5,51	3,72
22	5,7	3,44
23	5,77	3,22
24	6,18	4,28
25	6,3	3,18
26	8,3	1,99
27	8,68	1,36

Table 1: ***Data pairs used to construct the calibration curve. $\log_{10}$N is determined by the APC method and impedance detection time (IDT) is the time taken for an impedance change of the M-value at a 5 % threshold.***

Type of food	Intercept (*a*)	Slope (*b*)	S_e of estimation	R^2
Bitter chocolate	8.8219	-0.8545	0.671	0.8998

Table 2: ***The linear regression model between microbial population in bitter chocolate ($\log_{10}$N) estimated by the APC method and impedance detection time (IDT) measured by the impedance method with BacTrac™ 4000 (Se: there is 95.5% certainty that the actual value of y will lie within ± 2*Se (2*0.671=1,34) of the estimated value of y that is ý ± 1,34)***

Discussion and conclusions

The regression line equation and its parameters confirm that impedance detection time (IDT) is inversely proportional to the log number of microorganisms in the chocolate mix used as sample. This calibration curve can be used to measure microbial counts from impedance detection time of a specific sample (of the same type of chocolate). Total microbial count is obtained by calculating the antilogarithm of the y estimation in the equation y=a+bx.

The microbiological limits for chocolate for the aerobic plate count (APC) which are proposed range between 10^4 to 10^6 per g (14,15). Especially, the tolerable value in Switzerland[16] is 10^5/g and $5x10^4$/g in Germany (17). According to the regression line equation of this model, detection time for a sample containing about 10^4 to 10^6 microorganisms/g ranges between 3.3 to 5.6 hours. Therefore, this method offers the advantage of a rapid screening of chocolate samples used as finished product or raw material to reveal if microbial criteria are met. The minimum limit of microbiological standards for chocolate which is 10^4 microorganisms/g can be detected in less than 6 hours with impedance measurement, compared to the 48 hours required for the APC method.

However, we would like to point out that the main difficulty in establishing the calibration curve was to obtain samples of chocolate of a wide range of microbial levels. On the other hand, most microbial contamination of the chocolate mix occurs (according to our experience) either from the raw materials used or from a leakage of water in the heating systems (pipes, pumps, etc) that results in the increase of the a_w of the product and the consequent augmentation of the initial numbers of the endogenous microflora. Therefore, we decided to assimilate this phenomenon by incubation of chocolate suspension to achieve

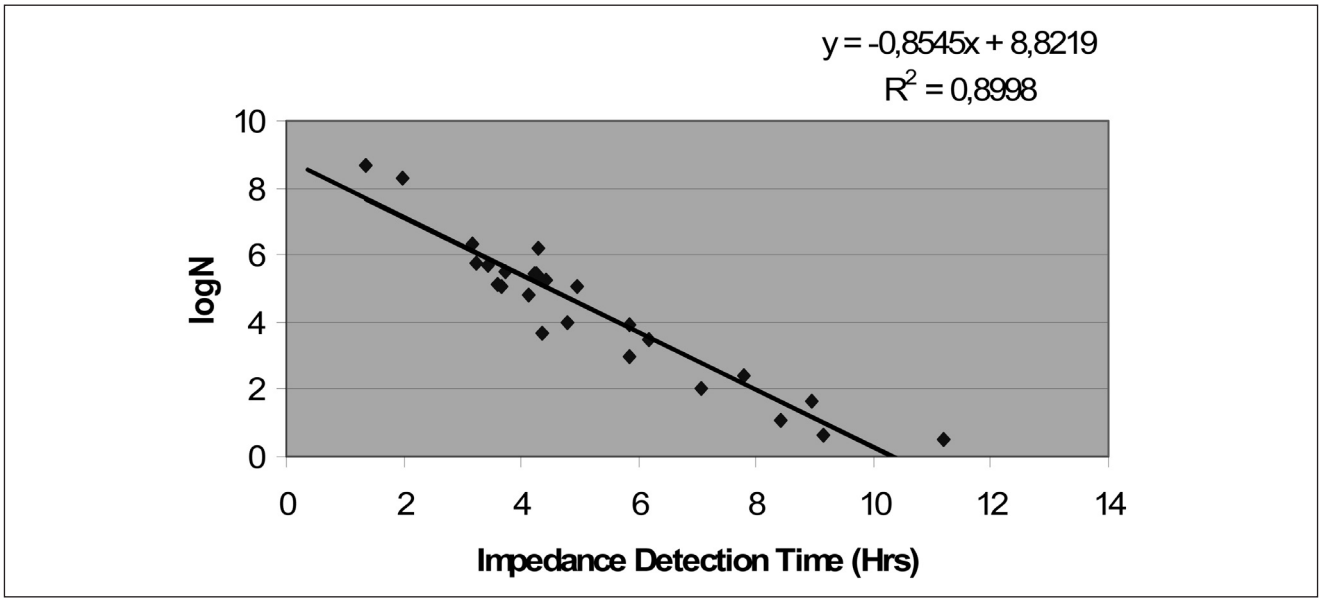

Figure 1: Calibration Curve of the microbial population (log_{10} N) and the IDT (in hrs).

proliferation of the existing microorganisms. It should be noted here, that because the samples used were artificially contaminated this might result in a shorter lag phase of microorganisms than in naturally contaminated chocolate mix.

As a general conclusion impedance measurement which is more rapid, automated and less laborious, could be used as an alternative method for the early detection of microbial levels in this specific mix of chocolate. Finally, it should be pointed out that impedance measurement is closely dependent on the composition of the food being tested. As a consequence, variations in the ingredients of a food may result in differences in the pattern of impedance change and therefore a readjustment of the calibration curve should be performed for each type of chocolate formulation.

Aknowledgements

This research was entirely supported by YOTIS SA. a Greek leading Food Nourishing Company

References

(1) *D.Y.C. Fung*, Rapid Methods and Automation in Microbiology. *Comprehensive reviews in Food Science and Food Safety. 1* (2002) 3-22.

(2) *R. Bossuyt, G. Waes*, Impedance measurements to detect post pasteurization of pasteurized milk, *J.Food -Prot. 46* (1983) 622-624.

(3) *I.D. Ogden, D.C. Cann*, A modified conductance medium for the detection of Salmonella spp., *J. of Appl. Bacteriology. 63* (1987) 459-464.

(4) *J.D. Philipps, M.W. Griffiths*, An electrical method for detecting *Listeria* spp., *Letters in Applied Microbiology. 9 (*1989) 129-132.

(5) *K.O. Colquhoun, S. Timms, C.R. Fricker*, Detection of *Escherichia coli* in potable water using direct impedance technology, *Journal of Applied Bacteriology. 79* (1995) 635-639.

(6) *T. Deak, L.R. Beuchat*, Evaluation of indirect conductance method for the detection of yeasts in laboratory media and apple juice, *Food microbiology. 10* (1993) 255-262.

(7) *L. Curda, M. Plockova, E. Svirakova*, Growth of *Lactococcus lactis* in the presence of nisin evaluated by impedance method, Institute of Chemical Technology, Prague, (1995).

(8) *K., Futschik, A. Pfuetzner, A. Doblander, H. Asperger*, Automatical registration of microorganism growth by a new impedance method, *Abstr. Int. Meet. Chem. Eng. & Biotechnol, Achema. 88* (1988) 3.

(9) *S.H. Flint, J.D. Brooks*, Rapid detection of *Bacillus stearothermophilus* using impedance-splitting, *Journal of Microbiological Methods. 44* (2001) 205-208, 116.

(10) *M. Fontana, S. Busiello, S. Bisotti, G. Dallorto, G. Unger, G. Schwameis, U. Juterschnig, B. Unger, H. Masaniger, M. Schinkinger,* Rapid enumeration of clostridial spores in raw milk

samples using an impedimetric method, *Journal of Rapid Methods and Automation in Microbiology. 10* (2002) 107-10) 11.

(11) *J. Dupont, F. Dumont, C. Menanteau, M. Pommepuy*, Calibration of the impedance method for rapid quantitative estimation of *Escherichia coli* in live marine bivalve mollusks, *Journal of Applied Microbiology. 96* (2004) 894-902.

(12) *G. Kroyer, K. Futschik*, The Impedance Splitting method for microbiological analysis of food Preservatives, *Reprint-Biotech Lab International. 2* (1997) 3-4.

(13) *C. Gerolimatou, A. Batrinou, J. Tsaknis, V. Spiliotis*, Comparison of the Impedance Splitting Method to the Agar Dilution Method for the estimation of the antimicrobial activity of Food Preservatives, *Journal of Rapid Methods and Automation in Microbiology. 12* (2004) 259-267

(14) IFST, Development and Use of Microbiological Criteria for Foods, Institute of Food Science and Technology (1999) (UK), London.

(15) *H. Pivnick, D.A. Gabis*, "Confectionery products" in *Compedium of Methods for the microbiological examination of foods,* 2nd Edition, Washington D.C., Marvin L. Speck, Editor, American Public Health Association (1984).

(16) Verordnung über die hygienisch-mikrobiologischen Anforderungen an Lebensmittel, Gebrauchs- und Verbrauchsgegenstände, Schweiz, 26.06.1995

(17) Lebensmitteltechnik , Richt- und Warnwerte für Schokoladen und Kakaopulver der DGHM, Lebensmitteltechnik, *31* (1999) 62–63

Adress of the Authors:

*A. M. Batrinou, E. D. Katsogiannos, E. N. Koustoumpardis, V. K. Spiliotis**

Department of Food Technology,
Technological Educational Institute of Athens,
Ag. Spiridonos str., 12210 Egaleo,
Athens,
Greece

Eingelangt am: 03.02.2005
Akzeptiert am: 11.03.2005

**to whom correspondence should be addressed*
tel. 0030210-5385530
e-mail: vspili@teiath.gr

ernährung

ÖSTERREICHISCHE ZEITSCHRIFT FÜR WISSENSCHAFT, TECHNIK, RECHT UND WIRTSCHAFT

Offizielles Organ der Österreichischen Gesellschaft für Ernährung (ÖGE) und ihrer Sektionen und Zweigvereine, des Fachverbandes der Nahrungs- und Genussmittelindustrie Österreichs, des Schutzverbandes der österreichischen Lebensmittelindustrie.

Herausgeber:
Fachverband der Lebensmittelindustrie
A-1030 Wien, Zaunergasse 1-3

Baurat h. c. Ziv.-Ing. Dipl.-Ing. Otto Riedl
A-1190 Wien, Felix-Mottl-Straße 50

Wissenschaftlicher Beirat:

Generaldirektor Univ.-Prof. Dr. iur. et rer. pol. W. Barfuß
Univ.-Prof. Dipl.-Ing. Dr. nat. techn. E. Berghofer
Univ.-Prof. Dipl.-Ing. Dr. nat. techn. Dr. h. c. E. Brandl
Vizepräsident des OGH Hon.-Prof. Dr. K. Brustbauer
Univ.-Prof. Dr. med. P. H. Clodi
Univ.-Prof. Dr. med. W. Druml
Univ.-Prof. Dr. agr. I. Elmadfa
Univ.-Prof. Dr. med. J. M. Hackl
Univ.-Prof. Dr. med. K. Irsigler
OR Dr. L. Jirovetz
Univ.-Prof. Dipl.-Ing. Dr. techn. Dr. h. c. H. Klaushofer
MR Dr. P. Kranner
Univ.-Prof. Dr. med. vet. J. Leibetseder
Hofrat Dr. iur. W. Olscher
Hon.-Prof. Dr. iur. K. Smolka
ao Univ.-Prof. Dr. I. Steiner
Univ.-Prof. Dr. med. R. Wenger

Chefredakteur: Dr. Michael Blass

Redaktion „Wissenschaft": Dipl.-Ing. Dr. Udo Pechanek

nutrition

AUSTRIAN JOURNAL FOR SCIENCE, TECHNOLOGY, LAW AND ECONOMY

Österreichische Spirituosenzeitung

FÜR INDUSTRIE, GEWERBE UND HANDEL FACHBLATT FÜR DIE SPIRITUOSENERZEUGUNG, WEIN- UND OBSTBRENNEREIEN, FRUCHTSÄFTE UND SEKTERZEUGUNG SOWIE GÄRUNGSESSIGE

Offizielles Organ des Verbandes der Spirituosenindustrie und des Schutzverbandes Österr. Spirituosen-, Sekt- und Fruchtsafthersteller

Redaktion: Dr. Bruno Mayer

Verleger:
Fachzeitschriftenverlagsges. m. b. H.
A-1030 Wien, Schwarzenbergplatz 6
Telefon (01) 715 31 93 Telefax (01) 715 48 19
E-Mail: ernaehrung@dielebensmittel.at
Geschäftsführer: Dr. Bruno Mayer

Die „ernährung" (nutrition) – ISSN 0250-1554 – erscheint elfmal jährlich.

Jahresabonnement Inland € 72,–
Einzelpreis Inland € 10,–
einschließlich 10 Prozent MWSt.
Jahresabonnement Ausland € 90,–
Einzelpreis Ausland € 11,–
Anzeigen: Es gilt Tarifblatt 2004.

Anzeigenannahme:
A-1030 Wien, Schwarzenbergplatz 6
Telefon (01) 715 31 93 Telefax (01) 715 48 19
E-Mail: ernaehrung@dielebensmittel.at

Hersteller: Ueberreuter Print und Digimedia, 2100 Korneuburg