UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
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INTELLIGENT PACKAGING: FEASIBILITY OF USING A BIOSENSOR COUPLED TO A ULTRA HIGH FREQUENCY (UHF) RADIO FREQUENCY IDENTIFICATION (RFID) TAG FOR TEMPERATURE MONITORING

Rio de Janeiro

2017

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The Doctoral School GAIA: Agro Resources, Processes, Foods, Byproducts and the Research Unit of Agro-Engineering of Agro Polymers and Emerging Technologies, University of Montpellier
Specialty: Biomaterials

And

Thesis presented to the Graduate Program in Technology of Chemical and Biochemical Processes, School of Chemistry, Federal University of Rio de Janeiro as partial requirement for obtaining the title of Doctor in Technology of Chemical and Biochemical Processes

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Rio de Janeiro, Brazil / Montpellier, France

2017

CATALOGUING DATA

Silva, Fernando Teixeira
S363i Intelligent packaging: feasibility of using a biosensor coupled to a UHF RFID tag for temperature monitoring / Fernando Teixeira Silva. -- Rio de Janeiro, 2017.
135 f.

Orientadora: Verônica Maria de Araújo Calado.
Tese (doutorado) - Universidade Federal do Rio de Janeiro, Coordenação dos Programas de Pós-Graduação em Engenharia, Programa de Pós-Graduação em Engenharia Química, 2017.

1. Biosensores. 2. Propriedades elétricas de proteínas. 3. Gelatina. 4. Cozimento de carnes. 5. Identificação por rádiofrequência (RFID). I. Calado, Verônica Maria de Araújo, orient. II. Título.

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“The answer is inside you”
Alexandre Miglioranza

“Face the heat, dare to
beat the system »
Bob Hartman

DEDICATION

To Jesus whose peace is deeper than all knowledge

To Ellen my dear wife

To Rebecca and Miguel my wonderful children

ACKNOWLEDGEMENTS

I would like to thank God for giving me the strength and hope for the challenges of the life.

Many thanks to Ellen, my wife, who supported my decision to start my Ph.D despite the significant changes it caused in her live. You have provided stability to our family by taking charge of our home and our children. I would like also to thank Rebecca and Miguel for their understanding and encouragement to the conclusion of this project, you were always my motivation. All of you were patient and at the same time inspiration for this stage of my life. It was essential to have this beautiful family in my daily life. You also made the experiments with me, you were also sleepless sometimes, sad, happy and you came until the end with me. The doctorate is also yours. It is a victory of our family. I love you so much.

I would like to thank the whole family and Brazilian friends and the new and good French friends as well. I already had a treasure and now it got bigger and international. Their support was a relief!

Annelise you have helped me a lot with the translations to French. I really appreciate it. Thank you very much.

I would like to thank Regina Lago and Lourdes Cabral that, being head of Embrapa Food Technology, has allowed the realization of this PhD. Having your names mentioned, I would like to extend my thanks to the whole company. However, I would like to highlight André and Marcos Moulin for the help in the drawings and the friends of technology transfer who have bravely overcome my absence during this period.

I also thank Amauri, my academic counselor, Esdras and Angela. All of you have helped me in the worst moments of my thesis.

Veronica Calado, my dear adviser, she has welcomed me as a student and we have become friends. She was fantastic as an advisor because she got directly involved in the execution of the work and has made the things happen. Besides supervising me, you have always pushed me further to scientific understanding. I would like also to thank you for the touches, your dedication and for all help to conclude this thesis.

Thanks Rosana for the analysis of TG and DSC, Alfredo for the statistical analysis and Filipe, my companion of PhD and *peixeira*. I am grateful to Prof. Andrea and Prof. Karen as well. Our conversations and brainstorming have helped me a lot on my work. Thanks Julio, you were always very quick to help me with administrative matters.

Thanks Nathalie Gontard my French advisor to have opened the doors to welcome me. I am proud to have worked with you. I have learned many things necessary for this project but also for my professional life.

With much gratitude, I mention Carole Guillaume, her commitment and dedication were essentials at the beginning of the work in France. You were always helpful even before arriving in France. Among your many virtues, I would like to highlight the safety that is to work with you.

It is difficult to find words to talk about Brice, everything would not be enough; without him the doctorate would not exist. You were my lighthouse. I am proud to join the Brice academy. You are the best, my friend!! Thank you all IES, what a wonderful place to work! Thanks Arnold, Mamadou, Tatiana for the friendship and for helping me better understanding the ways of electronics.

Thanks Prof. Philippe Papet for allowing and teaching me how to use the Impedance Analyser Material. You were always very kind. Thanks Antero for the information related to meat processing.

Thanks Frédéric Fernandez, you have helped me not only with SEM analysis, but also to understand the results. I would like to thank Brigitte, Michel, Christophe, Laurence and Carole Villard. Besides the attention, all of you make my daily live easier.

It was great getting along with my friends of PhD, we have built a beautiful friendship. It was easier with you. I emphasize those who were with me from the beginning: Beatriz, An, Asma, Claudia, Maud, Gregory, Ali, Filippo, Fabien, François, Aida and Chutima. I will miss all of you.

Thanks Patricia Havelange, Claudine Charpentier and Estelle Monteil for your patience with me and my poor French. I would like to thank you also for your help to comply the rules of the University of Montpellier.

I would like to thank Valérie, Hélène, Pascale, Emmanuelle, Stéphane, Alain and Sylvie as well. You made me feel welcome and you were available to answer any of my questions.

It was amazing the manner in which I was received by Valérie Micard, Hugo de Vries and Alain Foucaran. Even though the many responsibilities they were always attentive and they had words of encouragement.

RESUMO

Embalagem inteligente (IP) é uma tecnologia emergente baseada na comunicação das embalagens e tem a Identificação por Radio Frequency (RFID) como conceito mais promissor. RFID se refere a tecnologias e sistemas que usam ondas de rádio para transmitir e identificar de forma única e / ou rastrear objetos com informações precisas em tempo real.

A inovação da presente tese é baseada nas propriedades elétricas (capacitância, permissividade real e fator de perda) da proteína isolada de soja, gelatina e caseinato de sódio, visando a sua utilização como sensor de temperatura acoplado a etiquetas RFID. As variáveis ambientais foram temperatura (20°C a 80°C) e umidade (90% HR) que são normalmente utilizadas no cozimento industrial de carnes. A gelatina apresentou maior sensibilidade. Após esta primeira parte, o trabalho prosseguiu tendo as seguintes etapas:

• Analisar o impacto da espessura do filme de gelatina na capacitancia elétrica e a determinação de vários parâmetros, tais como sensibilidade, histerese e repetibilidade;

• Analisar etiquetas RFID cobertas por gelatina a 90% de RH e variação de temperatura (20°C até 80°C) em condições piloto. O impacto sobre a faixa de leitura teórica foi analisado.

O potencial de gelatina como sensor foi demonstrado em amostra com espessura de 38 μm na qual a capacitância foi estável (20°C a 80°C) na faixa de Frequência Ultra-Alta (300-900 MHz). A amostra a 125 μm sofreu colapso eletrotérmico entre 60-80°C. Para superar este fenômeno, a frequencia de 600 MHz foi aplicada. O equilíbrio entre a espessura e a frequência, deve ser considerado para aumentar a sensibilidade que foi 0,14 Pf/°C (125 μm a 600 MHz) e 0,045 Pf/°C (38 μm a 868 MHz), influenciando os resultados da simulação do cozimento da carne. A reutilização do mesmo sensor levou à perda de sensibilidade devido a redução de massa. A etiqueta RFID, coberta pelo filme de gelatina em toda a área da antena, apresentou melhores resultados porque foi capaz produzir diferença significativa ($p<0{,}05$) na Faixa de Leitura Teórica (FLT) para 868 MHz, 915 MHz and 960 MHz. Também neste layout, a FLT foi igual para o valor de temperature de subida e descida (sem histerese) na zona crítica (60°C a 80°C e 60°C a 20°C) a 915 MHz. Os resultados abrem possibilidade para uma nova concepção de sensor de temperatura baseado em biomateriais, de baixo custo e renováveis, acoplados a etiquetas RFID passivas em embalagem inteligente.

Palavras-chave: Gelatina; Propriedades Elétricas, Biosensor, RFID, Sensor de Temperatura.

ABSTRACT

Intelligent packaging (IP) is an emerging technology based on the communication function of packages. Radio frequency Identification (RFID) is considered the most promising concept of IP. RFID refers to technologies and systems that use radio waves (wireless) to transmit and uniquely identify and/or track objects with accurate information in a real time.

The present thesis is based on an innovative study of the electrical (capacitance) and dielectric properties (real permittivity and loss factor) of soybean isolated protein, gelatin and sodium caseinate aiming at their use as a sensor of temperature coupled with RFID tags. The environmental variables were temperature (range from 20°C up to 80°C) and humidity (90% RH) that are normally used for meat cooking. Gelatin was the most sensitive sensor. After this first part, several steps have been set up:

- Analysing the impact of gelatin film thickness on electrical capacitance and the determination of several parameters such as sensitivity, hysteresis and repeatability;
- The coating of gelatin on a RFID tag tested at 90% RH and variation of temperature (20°C up to 80°C) in a pilot condition. The impact on the reading range was analysed.

The potential of gelatin as a sensor was demonstrated at thickness of 38 µm and 125 µm. For the first case, the capacitance was stable at 20°C up to 80°C and at Ultra High Frequency band (300-900 MHz). Sample with 125 µm has suffered the electro-thermal breakdown between 60-80°C. To overcome this phenomenon, 600 MHz was applied. A balance between thickness and frequency should be consider to increase the sensitivity that was 0.14 pF/°C (125 µm at 600 MHz); this value was higher than 0.045 pF/°C (38 µm at 868 MHz) influencing the results in the simulation of meat cooking. Reuse of the same sensor has led to mass loss reducing the sensitivity. The feasibility of gelatin sensor-enable RFID tag was demonstrated. The tag covered by gelatin film in the whole antenna was suitable because it was able to deliver different Theoretical Reading Range (TRR) ($p<0.05$) for 868 MHz, 915 MHz and 960 MHz. At this layout also, the TRR was the same (without hysteresis) for the rising and descending temperature at the critical zone (60°C up to 80°C and 60°C up to 20°C) at 915 MHz. These promisor results open a window for new conception of temperature sensor based on biomaterial that confers advantages, such as low cost and eco-friendly property sought to be interfaced to passive RFID tags for intelligent packaging.

Key words: Gelatin; Electric properties, Biosensor, RFID, Sensor of temperature.

RESUME

L'emballage intelligent (EI) est une technologie émergente basée sur la fonction communicative des emballages. La radio-identification (RFID) est considérée comme le concept le plus prometteur de l'EI. La RFID fait référence aux technologies et systèmes qui utilisent les ondes radio (sans fil) pour transmettre et identifier de manière exclusive et/ou suivre des objets avec une information précise en temps réel.

Cette thèse est basée sur une recherche innovante des propriétés électriques (capacité, permittivité réelle et perte) de la protéine de soja isolée, de la gélatine et du caséinate de sodium, et vise leur utilisation comme capteurs de température, associés à l'étiquette RFID. Les variables étaient la température (20°C jusqu'à 80°C) et l'humidité (90% HR) qui sont normalement utilisées pour la cuisson de la viande. La gélatine s'est révélée être le capteur le plus sensible. Après cette partie, plusieurs étapes ont été menées :

- L'analyse de l'impact de l'épaisseur du film de gélatine sur la capacité et la détermination de plusieurs paramètres tels que la sensibilité, l'hystérésis et la répétabilité;
- La couverture de gélatine sur l'étiquette RFID, testée à 90% HR et à température variable (de 20°C à 80°C) en condition pilote. L'impact sur la bande de lecture a été analysé.

Le potentiel de la gélatine en tant que capteur a été démontré à une épaisseur de 38 µm à laquelle la capacité était stable de 20°C à 80°C et à Ultra-Haute Fréquence (300-900 MHz). L'échantillon de 125 µm a subi une dégradation électrothermique entre 60°C et 80°C. Pour surmonter ce phénomène, 600 MHz ont été appliqués. Un équilibre entre l'épaisseur et la fréquence devrait être considéré pour augmenter la sensibilité qui était de 0,14 pF/°C (125 µm à 600 MHz) et 0,045 pf/°C (38 µm à 868 MHz), influençant les résultats lors de la simulation de cuisson de la viande. La réutilisation du même capteur a conduit à une perte de masse réduisant la sensibilité. L'étiquette RFID couverte d'un film de gélatine sur l'antenne a pu donner de différence significative (p <0,05) dans la Bande de Lecture Théorique (BLT) à 868, 915 and 960 MHz. Également dans cette layout, la BLT a été la même pour la même température croissante et décroissante (pas de hystérésis) dans la zone critique (60°C à 80°C et 60°C à 20°C) à 915 MHz. Ces résultats ouvrent une porte à une nouvelle conception de capteurs de température basés sur les biomatériaux, renouvelable at à faible coût, couplé avec des étiquettes RFID passives pour l'emballage intelligent.

Mots-clés : Gélatine, Propriétés électriques, Biocapteur, RFID, Capteur de température.

SUMARY

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

TABLE OF SYMBOLS

A	Electrode surface
μm	Micrometre
Auto ID	Automatic Identification
a_w	Water activities
BLT	Bande de Lecture Théorique
BUE	Blank uncoated electrode
C	Capacitance
CCP	Critical Control Point
cm	Centimetre
$CONH_2$	Amide
COOH	Carboxylic acid
d	Distance between two electrodes
DNA	Deoxyribonucleic acid
DSC	*Differential Scanning Calorimetry*
ε'	Real permittivity
ε''	Imaginary component
ε_0	Vacuum permittivity
ε_r	Relative permittivity
FLT	Faixa de Leitura Teórica
GEL	Gelatin
GHz	Gigahertz
GRAS	Recognized as a safe
H_2S	Hydrogen sulfide
HF	High Frequency
IDC	Interdigital electrodes
IP	Intelligent Packaging
IRT	Infrared Thermography
KNPs	Key Noise Parameters
LF	Low Frequencies
m	Metre
MHz	Megahertz
MR	Microwave Radiometry

mV	Millivolts
NH_2	Amine group
^{o}C	Celsius degree
OH	Hydroxide
pF	Picofarad
PVA	Polyvinyl acetate
RFID	Radio frequency identification
RH	Relative humidity
s	Second
SCA	Sodium caseinate
SEM	*Scanning Electron Microscopy*
SH	Sulfhydryl groups (–SH)
SIP	Soybean isolated protein
TC	Thermocouples
T_g	Glass transition temperature
TGA	Thermogravimetric Analysis
TLCs	Thermochromic liquid crystals
TRR	Theoretical Read Range
TTI	Time temperature indicator
UHF	Ultra High Frequency
USN	Ubiquitous Sensor Network
VHF	Very High Frequency
w/v	Weight / volume
WSN	Wireless Sensor Networks

INTRODUCTION

Biopolymers are abundant, renewable and used in a wide range of technical applications. Because of the film forming ability, they are potential substitutes to synthetic materials used in food preservation and food packaging (Bergo and Sobral, 2007, Mudhoo, 2011, Landi et al., 2015).

Regarding to proteins, their humidity and temperature dependences, mainly studied on gas and vapor transfer properties, permit the use in the field of selective materials, active materials, and self-adjusted material.

The complexity and inhomogeneous structure of proteins together with different origins make difficult to determine their electrical properties (Pitera et al., 2001, Berkowitz and J. Houde, 2015, Marzec and Warchoł, 2005). It is reported that the ability to store energy (real permittivity (ε') and to dissipate electrical energy (imaginary component (ε'') define the biopolymers as non-ideal capacitors (Ahmed et al., 2008), turning mandatory to take the electrical properties at frequencies and ambient of interest. Besides, the capacitance dependence on the external stimulus makes the sensors easier to implement, and their use has become extended (Venkatesh and Raghavan, 2004, Büyüköztürk et al., 2006, Rittersma, 2002).

There are in the literature researches on electrical properties of soybean isolated protein (SIP) (Ahmed et al., 2008), caseinate (Mabrook and Petty, 2003) and gelatin (Kanungo et al., 2013, Kubisz and Mielcarek, 2005, Clerjon et al., 2003, Landi et al., 2015). However, the electrical properties of proteins have been largely studied when dispersed into solution, but the literature is relatively scarce concerning protein based material, which is worse when considering variation with temperature and/or humidity on a large frequency range. This dependence might be of interest in the field of intelligent packaging biosensor to indicate temperature and/or humidity changes featuring an innovative and unusual application of biosensor that usually converts a biological response into an electrical signal.

Temperature measurement in the food safety is a Critical Control Point (CCP). Thermocouples are widely used because of its reliability and low cost (Zell et al., 2009) but it generate large degree of uncertainty and local limited information (Wold, 2016, Guérin et al., 2007). These features have been opening windows for new methods.

Combining temperature sensor or indicator with an RFID tag can be the best choice for products in the chilling chain (Wan and Knoll, 2016). It confers the wireless characteristics representing the next generation on monitoring temperature. For this purpose, RFID technology is recognized as a new generation of smart RFID tag for intelligent food packaging and notorious advantage by reduction and simplification in wiring and harness (Badia-Melis et al., 2014, Kim et al., 2016b). The researches combining Time Temperature Indicator (TTI)-RFID in the cold chain and examples of wireless sensor (Dwivedi and Ramaswamy, 2010) reinforce the potential use of RFID in processing steps in the food industry.

Our research group has been studying the electrical properties of biopolymers to investigate how these properties depend on the temperature and humidity (Bibi et al., 2016a). Proteins are good candidates because of their sustainability coming from renewable resources and from by-products. Another advantage is the potential use for both temperature monitoring and/or humidity and food quality makers (as biosensor).

Combination of biology and electronics is a promise for on-line measurements of important process parameters and microbial detection (Ramaswamy et al., 2007). Our proposal is the use of biopolymers as innovative sensors of temperature. The objectives of this thesis were: part 1: to prepare a review about the stat of art of the sensors of temperature; part 2: to select the biomaterial based on the electric capacitance; part 3: (1) to study the influence of the layer thickness on the electrical capacitance sensitivity, (2) to evaluate its application under meat cooking protocol, and (3) to evaluate its stability for continuous use of the same sensor; part 4: study the RFID performance of gelatin as sensor of temperature.

TECHNICAL CONTEXT

The new technology developed within this project will permit to monitor time-temperature history of food product subjected to heating/cooling treatments when packed. Variables for meat cooking, based on ham production, have been considered because it is noble and popular, but most of all because:

- It is processed after packing and
- Its shelf life is correlated to microbial spoilage that occurs when temperature is not properly managed upon heating and cooling steps.

Then, it constitutes a good model food to develop the new technology and it will be possible to apply all the knowledge and skills gain within this project to other line products that are produced under similar conditions of heating/cooling treatment, under package (step common for most products). Technical aspects are indicated below:

- During heating, the temperature monitoring will be carried out by replacing thermocouple sensor with a bio-based sensor coupled to RFID chip. The advantage of this new RFID tag is that it can be applied on each pieces of packed (if wanted) ham whereas thermocouples are pushed in only some items. This will permit also the better control of the oven cooking ham by placing the tags in different position inside it allowing a uniform and effective control of the heating processing.

- Current thermocouples are time-temperature indicators (indicating the history of the product instead of its quality), but the sensor-enable RFID tag proposed within this project, may be also a direct indicator of food quality and safety. It means that it will not only inform whether temperature is properly manage or not during heating, but also diagnose if a wrong temperature management affects the quality and safety of the product upon both heating and cooling (this second possibility was not explored in this thesis). This will permit:

    - To better control and monitor the process of every ham piece at each stage of the process (from the heating to the cooling);
    - To quickly adjust and propose remedial actions regarding the heating and cooling processes considered as Critical Control Point (CCP).

SCIENTIFIC CONTEXT

Proteins are the natural polymers considered in the project to elaborate sensing bio-material for both sustainability and applicability concerns. They come from renewable resources (then so-called agro-polymers) and some can be even by-products from food industry. They are good candidates to develop the sensor that will be further coupled with RFID as they exhibit:

- Good film forming ability;
- Electrical properties already demonstrated to determine molecular weight in electrophoresis methodology;
- Temperature dependence of their electrical properties used to assess glass transition temperature in dielectric analysis experiments;
- They are heteropolymers, i.e., they have the ability to interact with a large range of molecules by different interactions (H-bonding, hydrophobic interactions, covalent in some case, etc.).

Electrical properties of the sensing material are key parameters to further design RFID tag, once the proper reading of RFID tag is based on electrical properties of the system. The project will bring new knowledge on the temperature dependence of electrical properties of protein based films in different temperatures and humidities conditions.

Particular attention will be paid to assess electrical properties on a large range of radio frequencies, from 1 kHz to 1 GHz for further coupling with passive RFID tags, in agreement with the foreseen application.

The studies were conducted at the Joint Research Unit Agropolymers Engineering and Emerging Technologies (UMR IATE), Institute of Electronics and Systems (IES) that have developed a specific device to assess electrical properties of materials (based on the use of inter-digitate electrodes) on a large range of frequencies, both at University of Montpellier and at Testing Laboratories Composites and Thermal Analysis of the LADEQ at Federal University of Rio de Janeiro.

ORGANIZATION OF MANUSCRIPT

The manuscript was organized in 6 parts. The first is a summary in French and English of the project that meets requirement of the University of Montpellier named part zero (P0). The others 4 parts were structured under format of 4 publications and the last one related to general perspectives and references used within this project.

Part 0: Summary of the thesis.

Part I: Literature review.

Part II: Selecting of sensing biomaterial

Part III: Biomaterial evaluation

Part IV: Sensor-enable RFID tag

Part V: General Discussion

Part 0: Résumé du projet

Emballage intelligent: faisabilité de l'utilisation d'un biocapteur couplé à un tag RFID UHF pour le suivi de la température.

1. Introduction

Les biomatériaux sont abondants, renouvelables et utilisés dans une large gamme d'applications techniques. Grâce à leur capacité à former un film, ils sont de potentiels substituts aux matériaux synthétiques utilisés dans la conservation des aliments et l'emballage alimentaire (Bergo et Sobral 2007, Mudhoo 2011, Landi, Sorrentino et al. 2015).

En ce qui concerne les protéines, leur dépendance à l'humidité et à la température, principalement étudiée sur les propriétés de transfert de gaz et de vapeur, permet leur utilisation dans le domaine des matériaux sélectifs et actifs.

La complexité et la structure inhomogène des protéines associées à différentes origines rendent difficile la détermination de leurs propriétés électriques (Pitera, Falta et al. 2001, Marzec et Warchol 2005, Berkowitz et J. Houde 2015). On a constaté que la capacité d'emmagasiner de l'énergie (permittivité réelle (ε') et de dissiper l'énergie électrique (composante imaginaire (ε'')) définissait les biomatériaux comme des condensateurs non-idéaux (Ahmed, Ramaswamy et al. 2008), rendant obligatoire le fait de prendre les propriétés électriques aux fréquences et ambiant d'intérêt. De plus, la dépendance de capacité électrique sur le stimulus externe rend les capteurs plus simples à mettre en œuvre et leur utilisation en a été prolongée (Rittersma 2002, Venkatesh et Raghavan 2004, Büyüköztürk, Yu et al. 2006).

Les propriétés électriques des protéines ont été étudiées en grande partie lorsqu'elles étaient diluées dans une solution, mais la littérature est relativement rare concernant le matériau basé sur les protéines, et d'autant plus si l'on prend en considération la variation de température et/ou d'humidité sur une large gamme de fréquences. Cette dépendance peut être intéressante dans le domaine des biocapteurs de l'emballage intelligent pour indiquer les changements de température et/ou d'humidité présentant alors une application innovante et inhabituelle du biocapteur.

La mesure de température dans la sécurité alimentaire est un Point de Contrôle Critique (PCC). Les thermocouples sont largement utilisés pour leur fiabilité et leur faible coût (Zell, Lyng et al. 2009) mais ils génèrent un haut degré d'incertitude (Guérin, Delaplace et al. 2007, Wold 2016). Ces caractéristiques ont ouvert des portes à de nouvelles méthodes.

Combiner des capteurs de températures à une étiquette RFID peut être le meilleur choix pour les produits dans la chaîne du froid (Wan et Knoll 2016). Ceci vérifierait les caractéristiques sans fil qui représentent la génération future du contrôle de température. Dans ce but, la technologie RFID est reconnue comme une nouvelle génération pour l'emballage alimentaire intelligent (Badia-Melis, Garcia-Hierro et al. 2014, Kim, Shin et al. 2016). Les recherches qui combinent ITT et RFID dans la chaîne du froid et des exemples de capteur sans fil (Dwivedi et Ramaswamy 2010) renforcent l'utilisation potentielle du RFID dans les étapes de préparation dans l'industrie alimentaire.

Notre groupe de recherche a étudié les propriétés électriques des biomatériaux pour chercher à savoir comment ces propriétés dépendent de la température et de l'humidité (Bibi, Guillaume et al. 2016). Les protéines sont de bons candidats du fait de leur durabilité qui vient de ressources renouvelables et de produits dérivés. Un autre avantage est leur utilisation potentielle pour, à la fois, le contrôle de la température et/ou de l'humidité et les marqueurs de qualité alimentaire (comme biocapteurs).

Notre proposition est l'utilisation de biomatériaux en tant que capteurs innovants de température. Les objectifs de ce projet sont : en première partie, de préparer une analyse pour avoir un état actuel du capteur de température ; en deuxième partie, de sélectionner le biomatériau en fonction de sa capacité électrique ; en troisième partie, (1) d'étudier l'influence de l'épaisseur de la couche sur la sensibilité de la capacité électrique, (2) d'évaluer son application dans le protocole de cuisson de la viande et (3) d'évaluer sa stabilité pour une utilisation continue du même capteur ; en quatrième partie, d'évaluer la performance RFID de la gélatine comme capteur de température.

2. Matériel et méthodes

Les propriétés électriques ont été étudiées sous des températures allant de 20°C jusqu'à 80°C et à un taux d'humidité de 90% HR.

Partie 1: état actuel du capteur de température

Partie 2: évaluation des biomatériaux : gélatine, caséinate de sodium et protéine de soja isolée. Étape spécifique 2.4.

Partie 3 : Nous avons recouvert de gélatine (sélectionnée dans la première étape) des électrodes interdigitées (système IDC). Étapes spécifiques 2.5 et 2.6.

Partie 4 : Nous avons recouvert de gélatine des étiquetes RFID. Étape spécifique 2.9.

2.2 Préparation de la solution

10% w/v de protéine de soja isolée, de caséinate de sodium et de gélatine (Arfa, Chrakabandhu et al. 2007, Fakhoury, Maria Martelli et al. 2012, Helal, Tagliazucchi et al. 2012). Les bulles ont été enlevées sous vide.

2.3 Épaisseur

L'épaisseur moyenne a été mesurée par un micromètre digital portatif (0,001 mm). Pour l'épaisseur du marqueur RFID, un profilomètre a été utilisé.

2.4 Analyse thermogravimétrique

Des analyses thermogravimétriques (TG/DTG) ont été menées sur un modèle d'ATG Pyris 1, Perkin-Elmer. Le gaz porteur était de l'azote à un débit d'écoulement de 30 mL/min. La gamme de températures allait de 20°C à 80°C, à un taux de chauffage de 10°C/min. Les analyses ont été faites en double.

2.5 Calorimétrie différentielle à balayage (DSC)

L'analyse thermale de la gélatine a été menée dans un DSC de Perkin-Elmer, modèle Diamond, avec un appareil frigorifique externe (Intercooler II) et de l'azote comme système de purge de gaz, avec un débit d'écoulement de 20mL/min^{-1}. La gamme de températures allait de 25°C à 170°C, à un taux de chauffage de 10°C/min. Les analyses ont été faites en triple.

2.6 Microscopie électronique à balayage (MEB)

L'analyse MEB a été menée dans un FEI Quanta 200 FEG. Il était équipé de X-Max^{50mm2} (Détecteur au Silicium à Diffusion), fabriqué par Oxford Instruments.

2.7 Échantillons recouverts d'électrodes interdigitées

2.7.1 La préparation des échantillons

Nous avons recouvert de solutions la surface des électrodes interdigitées avec une référence de circuit de 1 GHz (Cirly, France), par l'applicateur de film Coatmaster 510 (Erichsen, Allemagne), suivi par une étape de séchage à température ambiante et humidité relative (environ 25°C et 50%, respectivement). Une électrode vide a également été utilisée comme référence.

2.7.2 La détermination de la capacité électrique

La capacité électrique a été déterminée suivant le modèle Gervogian (Wang, Chong et al. 2003). Un analyseur d'impédance HP 4191A RF a été utilisé, à une gamme de fréquences de 300 à 900 MHz et a été lié à des électrodes interdigitées par un câble coaxial semi-rigide SMA (Amphenol Connex, France) et au connecteur coaxial SMA 500HM Solder SMA (Amphenol Connex, France). La température et l'humidité ont été contrôlées par une enceinte climatique (Secasi Technologies, France). Pour les mesures, l'échantillon a été conditionné à 20% et les températures ont varié (20°C, 50°C et 80°C). La procédure a été répétée à 55% et 90% HR. Le logiciel utilisé pour enregistrer les résultats était LabView (National Instruments).

Le procédé d'immobilisation des protéines sur la surface de l'électrode était de l'adsorption.

2.8 Échantillons autoportants

2.8.1 La préparation des échantillons

Les solutions ont été versées dans un récipient en plastique pour former un film humide, d'une épaisseur d'environ 0,8 cm. Après cela, elles ont été séchées à température et humidité ambiante. Elles ont été coupées avec une perforatrice afin d'obtenir des échantillons de 2 cm de diamètre (avant d'être complètement séchées). Un film en Teflon a également été utilisé comme référence.

2.8.2 Le conditionnement de l'humidité

La dépendance à l'humidité a été évaluée à deux différentes activités de l'eau (a_w). Les échantillons ont été placés dans des dessiccateurs avec des solutions saturées de sels de carbonate de potassium (111 g/100 mL) et de nitrate de potassium (47 g/100 mL). L' a_w a été prise chaque jour en triple (FA-st/1, GBX) jusqu'à stabilité. Pour la gélatine, le caséinate de sodium et la protéine de soja isolée, les valeurs de l'a_w stabilisée ont été de 0,82, 0,87, 0,85 pour le carbonate de nitrate et 0,40, 0,38 et 0,43 pour le carbonate de potassium, respectivement.

2.8.3 La détermination de la capacité volumétrique

Les variables de réponse ont été la constance diélectrique et le facteur de perte, suivant l'équation :

$$C = \frac{\varepsilon_r \varepsilon_0 A}{d}$$

où *C* est la capacité, ε_r est la permittivité relative, ε_0 est la permittivité du vide ($8,85x10^{12}$ F/m), *A* est la surface de l'électrode et *d* est la distance entre les deux électrodes (épaisseur de l'échantillon). L'analyseur d'impédance matérielle HP 4291B a été utilisé d'1 MHz à 1,8 GHz. Cinq mesures ont été faites pour chaque échantillon.

2.9 La performance RFID

La performance des marqueurs RFID a été évaluée par le coupleur directionnel Voyantic (Finlande) 700-1200 MHz. Les étiquettes RFID recouverte de gélatine ont été positionnées sur une antenne à l'intérieur de l'enceinte climatique (Espec, Japon) pour contrôler à la fois l'humidité et la température. Le lien entre le support et le Voyantic a été établi par le câble RF et les mesures ont été enregistrées avec le logiciel de mesures Tagformance. Les variables de processus ont été : l'humidité (90% HR) ; temperature allant de 20°C jusqu'au 80°C.

Trois layouts ont été testées et comparées à une étiquette non revêtue: le film de gélatine a été appliqué sur (a) layout 1 - toutes les étiquettes sauf la zone de puce (b) layout 2 - la zone de puce et (c) layout 3- la boucle interne.

2.10 Analyses statistiques

Pour toutes les analyses statistiques, nous avons utilisé 5% comme niveau significatif et le logiciel Statistica, pour Windows, version 12.0 (Tulsa, USA). Toutes les données sont présentées comme des valeurs moyennes ± les écarts ordinaires. Pour la partie 1, nous avons utilisé un plan factoriel de 3^3 avec trois points centraux (Annexes 1 et 2).

3. Résultats et discussion

Partie 1 : État actuel

La température est généralement surveillée dans l'industrie alimentaire par un thermocouple basé sur des points de contrôle sur un faible nombre de produits qui génèrent un haut degré d'incertitude (Guérin, Delaplace et al. 2007, World 2016). Ces caractéristiques ont évoqué de nouvelles méthodes pour la surveillance de la température en se concentrant avant tout sur les mesures de température sans contact (non-invasives) (Eder, Becker et al. 2009, Knoerzer, Regier et al. 2009, Wan et Knoll 2016) et également sans fil.

Un capteur sans fil a été analysé par rapport aux thermocouples pour le contrôle de la température pendant la stérilisation d'aliments en conserve. Statistiquement, les deux capteurs n'ont pas donné de résultats différents ($P > 0,05$) en ce qui concerne les données de températures collectées (Dwivedi et Ramaswamy 2010).

Pour contrôler la température en post-traitement, la technique avancée est l'indicateur temps-température (ITT) incorporé avec une étiquette RFID, qu'il y aura une nouvelle génération de RFID intelligent pour l'emballage alimentaire intelligent (Badia-Melis et al., 2014, Kim et al., 2016b).

Nous n'avons utilisé aucune technologie RFID pour contrôler la température pendant les étapes de préparation. Les exemples de capteur sans fil et d'ITT-RFID sont les bases pour l'usage d'une utilisation innovante du RFID à fonction de capteur dans ce but.

Partie 2 : La sélection du biomatériau de détection

Selon l'analyse thermogravimétrique (TG), les altérations de températures du départ, prises juste après la première étape, étaient de 290,4 $\pm$ 11,9°C, 315,7 $\pm$ 3,6°C et 286,1 $\pm$ 0,6°C pour le caséinate de sodium (SCA), la gélatine (GEL) et la protéine de soja isolée (SIP), respectivement. Il n'y a pourtant pas de preuve d'altération à la température normalement utilisée pour la cuisson de la viande (80°C).

La capacité électrique de tous les biomatériaux était dépendante de la fréquence ; elle augmentait en même temps que la fréquence. Ce comportement était plus intense à un fort taux d'humidité (90% HR), ce qui correspond à la littérature (Ryynänen, 1995, Zhu et al., 2010).

La gélatine a montré une plus grande capacité ; quant à la protéine de soja isolée et au caséinate de sodium, leurs valeurs étaient similaires. Un poids moléculaire plus élevé de gélatine (300 kDa) (Figueiró et al., 2004) comparé à 20,2 à 81,4 kDa (Martins, 2005) pour la protéine de soja isolée, et 23 kDa (Gubbins et al., 2003) pour le caséinate de sodium, peut expliquer cette plus grande performance. Ce paramètre peut changer le comportement électrique (Kolesov, 1968).

Pourtant, la taille moléculaire de la gélatine (longueur de 300 nm et diamètre de 1,5 nm) (Figueiró et al., 2004) est bien plus petite que la longueur d'ondes à UHF (1 m-10 cm) (Sanghera and Thornton, 2007), menant à une dépendance des propriétés électriques seulement sur la forme (Ryynänen 1995).

La différence de formes moléculaires mène à des zones de différentes surfaces, des zones interfaciales et une polarisation interfaciale ; par conséquent, les propriétés diélectriques changent (Dang, Yuan et al. 2012). La caractéristique principale de la gélatine est son domaine hélicoïdal triple (Rest, Garrone et al. 1993), qui est stabilisé à la fois par les liaisons hydrogènes inter-chaînes et les molécules d'eau « structurales » (Sarti et Scandola 1995). La capacité de ses chaînes peptidiques planes à capter une grande quantité de molécules d'eau, permet d'utiliser leurs propriétés diélectriques intrinsèques (Sanwlani, Kumar et al. 2011). Même si le grand nombre de liaisons hydrogènes C=O…H-N limite la mobilité des groupes polarisés, ils sont très nombreux, ce qui est avantageux pour la polarisation (Ning, Wang et al. 2015).

La sensibilité aux conditions environnementales, telles que la température et l'humidité relative, est notée comme un facteur de restriction important en ce qui concerne les films parce que ce sont des matériaux hydrophiles et donc très sensibles à l'eau (Gennadios 1993). Leur sensibilité à la vapeur d'eau est notée comme le plus grand défi pour leurs applications pratiques (Potyrailo, Surman et al. 2011). Toutefois, dans notre travail, cette sensibilité a été appropriée puisqu'elle a changé les propriétés électriques indiquant la dépendance à l'humidité et à la température.

La température et l'humidité influencent toutes deux la capacité (Foucaran, Sorli et al. 2000). Mais ce n'est qu'à 90% HR, à cause de la polarisation de l'eau, que cette influence a été significative pour distinguer les températures. C'est une condition rêvée, puisque l'humidité utilisée pendant la cuisson de la viande est d'environ 90-95%. Concernant les 90% HR, le test de Fisher montre que, pour SIP, GEL et SCA, il y a eu une différence statistique significative ($p < 0,05$) seulement entre 20°C et 90°C. Cependant, seule la gélatine a pu avoir une différence statistique significative de capacité entre 20°C et 50°C, une gamme de températures commune dans les industries alimentaires.

Partie 3 : L'évaluation du biomatériau

Un matériau peut être ionisé et devenir conducteur, puisqu'aucun matériau diélectrique n'est un parfait isolant. Ceci a été observé sur des échantillons de capteur en gélatine de grande épaisseur (61 µm et 125 µm). Cette tendance peut être liée à la force diélectrique selon la théorie d'Artbauer et la théorie de la dégradation électrothermique (Li et al., 2015).

Ces théories sont toutes deux basées sur l'influence de la température, variable que l'on suppose être une explication à l'étape finale d'un processus de dégradation (Ho et Jow 2012). À 868 MHz, un effet conducteur a été constaté entre 60°C et 80°C et s'est arrêté après être revenu à 60°C.

La théorie d'Artbauer a été confirmée dans les mesures DSC de feuilles polypropylènes qui ont révélé une forte corrélation entre les phases de transitions structurales dans les mêmes régions de température, puisqu'elle montre des discontinuités dans la force de dégradation (Schneuwly et al. 1998). La même chose a été observée avec les propriétés électriques de la gélatine, puisque entre 60°C et 80°C la courbe de capacité a chuté et presque à la même fréquence de température où le T_g a commencé et s'est arrêté, dont la valeur extrapolée est 77,84 $\pm$ 0,13°C.

Ces deux théories mentionnent et expliquent la dépendance de la dégradation de la température qui est apparue seulement sur les échantillons les plus épais (61 µm et 125 µm), montrant que l'épaisseur est aussi une variable qui influence la dégradation électrothermique (Schneider et al., 2015). Ainsi, ces résultats mettent en avant la nécessité d'un bon équilibre entre épaisseur et fréquence pour une utilisation adéquate du capteur en gélatine. Suivant nos récentes recherches sur la permittivité réelle de la gélatine, 600 MHz a été la dernière fréquence avant d'atteindre la fréquence de résonance et c'était également la fréquence observée juste avant que ce phénomène ne se produise avec l'échantillon de 125 µm ; cette épaisseur a été choisie pour poursuivre les recherches, par rapport à celui de 38 mµ, puisqu'une sensibilité plus forte a été obtenue par rapport à 61 µm.

Les hystérésis pour 38 µm et 125 µm ont été évaluées. Les deux courbes (40-80°C et 80-40°C) pour 38µm étaient assez linéaires et, pour 125 µm, la linéarité de la courbe était convenable pour la température croissante, mais elle a changé pour la température décroissante. L'hystérésis maximum correspond à 6% de capacité à 40°C (125 µm), mais pour tous les autres points, elle était sous les 2%, présentant une boucle d'hystérésis étroite ; résultat soutenu par la littérature (Zhu et al, 2010). Dans nos tests précédents, nous avons observé la tendance à la stabilisation dans différents niveaux pour la même température (croissante et décroissante), généralement pour des échantillons plus épais que 50 µm.

Quant à la relation température vs capacité, l'échantillon de 125 µm présentait une courbe avec une pente plus forte, indiquant une plus grande sensibilité, qui a été calculée. La sensibilité pour l'échantillon de 125 µm était de 0,14 pF/°C, et celle de 38 µm était de 0,045 pF/°C, plus de 3 fois plus faible, ce qui montre qu'une épaisseur plus grande mène à une meilleure efficacité pour distinguer la variation de températures.

La plus grande sensibilité des échantillons les plus épais a été déterminante dans le test qui a simulé la température et l'humidité utilisées pendant la cuisson de la viande. Les capteurs en gélatine (38 µm à 868 MHz et 125 µm à 600 MHz) ont été testés en suivant les étapes de cuisson de la viande. On voit clairement que l'épaisseur la plus grande (125 µm) a conduit à des résultats plus perceptibles notamment dans les étapes de refroidissement, zone qui permet une bonne sécurité des aliments.

Si l'on prend en compte les produits prêts-à-manger tels que le jambon ou les saucisses, une étape de refroidissement est présentée, de 54,4 à 26,7°C, en seulement 1,5h et de 26,7 à 4,4°C en seulement 5h (USDA/FSIS 2001), étape essentielle pour réduire l'activité des microorganismes pathogènes (Mohamed 2008). Les deux échantillons ont pu montrer différentes capacités électriques ; pourtant, avec celui de 125 µm, le système est plus robuste.

Nous avons enquêté sur la répétabilité de capacité sur le même capteur (38 µm) en gélatine en l'utilisant trois fois à trois températures différentes (40°C, 60°C et 80°C), après l'avoir stocké à température ambiante (environ 25°C) et à un taux d'humidité environ égal à 60%. La valeur de capacité obtenue lors de la première mesure a servi de référence. En général, la réduction de capacité était d'environ 30% et 50% pour le deuxième et troisième fois, respectivement. La capacité obtenue chaque fois et à chaque température était le résultat de la moyenne des trois mesures (répétitions). Le coefficient of variation était en-dessous de 3%, montrant une robustesse des données. La réduction par rapport à la référence s'explique par la perte de matériel.

En effet, l'indicateur le plus important qui inhibe une utilisation continue du capteur n'est pas lié à une mesure électrique, mais à la réduction de sensibilité. Après un stockage à faible humidité, pour la troisième fois, les résultats étaient de 0,019 pF/°C, plus de deux fois inférieurs à la première fois (0,045 pF/°C).

Partie 4 : L’association du biocapteur et des étiquettes RFID

Le système RFID peut être utilisé à plusieurs bandes de fréquences, mais la plus utilisée est la Ultra-Haute Fréquence (UHF) et plus précisément les fréquences gérées par les règles des pays de façon individuelle : 868 MHz (Europe) et 915 MHz (États-Unis) (Sanghera, 2007). À UHF, il y a de nombreux avantages, tels que : un transfert de données plus rapide que les fréquences faibles et hautes, une distance de communication plus longue, des taux de données plus élevés, ainsi qu’une taille d’antenne plus petite sur les systèmes RFID (Sun et al., 2010).

L’absence de fréquence standardisée freine la mise en œuvre de la technologie RFID dans différentes applications (Sanghera, 2007). Il a été constaté, dans la littérature, qu’étant donné que 915 MHz et 868 MHz sont des fréquences proches, les caractéristiques de propagation et les conclusions peuvent également être étendues de l’une à l’autre (Angle et al., 2014). Cette approche n’est pourtant pas totalement applicable. En effet, nous avons noté une différence significative pour les layouts 1 et 2 ($p < 0,05$) entre les fréquences mentionnées ci-dessus, indiquant un comportement différent.

Le test de Fisher, en prenant en compte 5% de niveau significatif, a montré que la température, le layout, la fréquence et leurs effets d’interaction ont considérablement influencé les valeurs de radiofréquence. En comparant les résultats des trois layouts avec le layout de référence (sans film de gélatine), c’est clair qu'il y a influence de la gélatine sur la réponse RFID-capteur. Cependant, la meilleure performance du layout 1 (couverture de l'antenne entière) en termes de valeur absolue de la variation relative était exceptionnelle, confirmant l'importance de la couverture totale de l'antenne pour la surveillance de la température.

la Bande de Lecture Théorique (BLT) est un résultat d’une température donnée et une corrélation entre eux peut être établie ; il est souhaitable que la valeur de BLT pour une température croissante soit la même pour une température décroissante, signifiant qu’il n’y a pas d’hystérésis. Sur le layout 1 à 915 MHz, cette condition a été remplie à une zone de température critique nécessaire pour le contrôle efficace de pathogènes tels que *Clostridium perfringens* (60°C jusqu’à 80°C et 80°C jusqu’à 20°C). Même si l’absence d’hystérésis a été observée sur les layouts 2 et 3 à 915 MHz et 960 MHz, il n’y a pas eu de différence significative parmi les différentes températures (20°C, 40°C, 60°C et 80°C) ; ils ne conviennent donc pas pour le contrôle de la température à 915 MHz et 960 MHz.

Les fréquences normalement utilisées dans le système RFID à UHF fonctionnent avec une lisibilité réduite près de chargements de produits périssables riches en eau. L'eau absorbe l'énergie de la fréquence radio, réduisant la bande de lecture (Amador et Emond, 2010). En prenant comme référence la valeur normale de la bande de lecture pour une étiquette passive à 860-960 MHz, en-dessous de 10 m (Plos et Maierhofer, 2013), on peut voir que sur tous les layouts, les valeurs de BLT se trouvaient dans cette limite, montrant sa fiabilité. Cependant, l'influence de la température a été observée à 80°C puisqu'à cette valeur, le BLT était en-dessous de 10 m.

L'influence de l'eau peut être considérée comme un paramètre de bruit clés (PBC), puisqu'elle réduit la bande de lecture. La connaissance des PBCs est obligatoire dans les systèmes basés sur les ondes électromagnétiques, comme les RFID. Dans nos études précédentes (publications à venir), des essais ont été menés sous un taux d'humidité de 40% et 90% HR et l'influence de l'eau sur le BLT a sensiblement changé en fonction de l'humidité et de la fréquence. Pour 840 MHz, la variation de BLT a été d'environ 90% jusqu'à 130% pour 20°C et 60°C respectivement, et pour 868 MHz la variation a été d'environ 225%. Cependant, en considérant 80°C pour les deux fréquences, la variation de BLT a été d'environ 90% et 100% (840 MHz et 868 MHz, respectivement). Cette plus faible variation de BLT, comparée à 60°C, peut être liée à une influence de la transition vitreuse (Tg) de la gélatine. Par conséquent, outre l'influence de l'eau, il y a également une influence de la Tg sur le BLT. Ici, ni l'eau ni la Tg n'ont empêché pas la sensibilité sur les trois layouts, démontrant la robustesse de ce nouveau capteur pour surmonter ces PBCs.

Même si les résultats de chaque layout ont indiqué d'autres fréquences, 868 MHz et 915 MHz ont été les fréquences communes à tous ; ainsi, elles peuvent être utilisées comme références dans le choix du meilleur layout. Le layout 1, comparé au layout 2, était supérieur, étant donné qu'il y avait une différence significative dans les valeurs de BLT à la zone de température critique : en chauffant (60°C jusqu'à 80°C) et en refroidissant (80°C jusqu'à 20°C) pour toutes les fréquences. Ceci permet donc d'être plus flexible pour répondre aux différentes règles des pays pour savoir quelle fréquence adopter, entre 868, 915 et 960 MHz.

Outre le comportement sur l'hystérésis, la sensibilité à 915 MHz a également été remarquable par rapport aux autres fréquences (868 MHz et 960 MHz); elle peut être vue par l'inclinaison des courbes. L'erreur d'hystérésis était respectivement de 28% et 31% pour 868 MHz et 915 MHz; ces valeurs sont environ 3 fois comparées à 915 MHz dont la valeur était de 10% à 40°C qui se trouve à l'intérieur de la bande of variation acceptable. De plus, la sensibilité a été influencée par la bande de température et aussi pour la montée et la descente en température et par cette variable (sensibilité), on peut voir aussi les résultats exceptionnels à 915 MHz.

Basé sur 868 MHz, 915 MHz et 960 MHz, il peut conclure que le layout 1 est plus adapté puisqu'il permet de mieux distinguer la différence de valeurs de BLT parmi les températures. Il n'est pas possible d'utiliser le layout 3 à toutes les fréquences (868 MHz, 915 MHz et 960 MHz) parce qu'il n'y a pas eu de différence significative dans les valeurs de BLT parmi les températures.

Ces résultats montrent que la façon dont la gélatine a été placée sur l'étiquette influence clairement la valeur de BLT. En se basant sur les layouts 2 et 3, on peut en déduire que la couverture de la zone de la puce sur le premier a expliqué les meilleurs résultats. La couverture de l'antenne sur le layout 1 (sans que la zone de la puce ne soit couverte elle aussi) a été la caractéristique clé pour le capteur de température. Étant donné que l'antenne transmet l'information, il est raisonnable d'en restreindre le contact avec le matériau capteur (gélatine).

4. Conclusions

Les deux différentes techniques, l'échantillon autoportant et celui placé sur l'électrode interdigitée, ont été utilisées et elles ont toutes deux démontré l'influence de la polarisation de l'eau. La gélatine, du fait de sa forme moléculaire et de ses caractéristiques chimiques, a été le capteur le plus sensible. Quant à l'échantillon le plus épais (125 µm), la température a provoqué la dégradation électrothermique (vers la gamme de températures de 60°C jusqu'à 80°C), limitant l'utilisation à 868 MHz. Mais un équilibre entre cette épaisseur et la fréquence de 600 MHz a permis d'utiliser le capteur de gélatine avec une plus grande sensibilité. Cette combinaison, comparée à l'échantillon de 38 µm à 868 MHz, a eu pour résultats une plus

grande sensibilité et une meilleure condition pour distinguer les différentes températures utilisées normalement pendant la cuisson de la viande. La réutilisation du même capteur en gélatine plusieurs fois n’est pas recommandée, parce qu’elle réduit la sensibilité en raison de sa perte de masse après chaque utilisation. L’étiquette RFID couvert de gélatine à démontre de bonnes performances dans le contrôle de la température. Pour 868 MHz, 915 MHz et 960 MHz, le layout a été appropriée parce qu’il a pu donner différents résultats ($p < 0,05$) pour toutes les fréquences et il a été le seul (à 915 MHz) que la condition d'absence d'hystérésis a été remplie à une bande de température critique (60°C jusqu'à 80°C et 80°C jusqu'à 20°C). Nous obtenons de meilleurs résultats pour 915 MHz avec une hystérésis d'erreur de 10% et une sensibilité vingt fois plus importante que les autres fréquences (868 MHz et 960 MHz). En outre, le layout 1 à 915 MHz, indique l'utilisation potentielle de ce nouveau capteur pour les étapes de chauffage et de refroidissement lors de la cuisson de la viande.

Intelligent packaging: feasibility of using a biosensor coupled to a UHF RFID tag for temperature monitoring

1. Introduction

Biopolymers are abundant, renewable and used in a wide range of technical applications. Because of the film forming ability, they are potential substitutes to synthetic materials used in food preservation and food packaging (Bergo and Sobral, 2007, Mudhoo, 2011, Landi et al., 2015).

Regarding to proteins, their humidity and temperature dependences, mainly studied on gas and vapor transfer properties, permit the use in the field of selective materials, active materials, and self-adjusted material.

The complexity and inhomogeneous structure of proteins together with different origins make difficult to determine their electrical properties (Pitera et al., 2001, Berkowitz and J. Houde, 2015, Marzec and Warchoł, 2005). It is reported that the ability to store energy (real permittivity (ε')) and to dissipate electrical energy (imaginary component (ε'') define the biopolymers as non-ideal capacitors (Ahmed et al., 2008), turning mandatory to take the electrical properties at frequencies and ambient of interest. Besides, the capacitance dependence on the external stimulus makes the sensors easier to implement, and their use has become extended (Venkatesh and Raghavan, 2004, Büyüköztürk et al., 2006, Rittersma, 2002).

There are in the literature researches on electrical properties of soybean isolated protein (SIP) (Ahmed et al., 2008), caseinate (Mabrook and Petty, 2003) and gelatin (Kanungo et al., 2013, Kubisz and Mielcarek, 2005, Clerjon et al., 2003, Landi et al., 2015). However, the electrical properties of proteins have been largely studied when dispersed into solution, but the literature is relatively scarce concerning protein based material, which is worse when considering variation with temperature and/or humidity on a large frequency range. This dependence might be of interest in the field of intelligent packaging biosensor to indicate temperature and/or humidity changes featuring an innovative and unusual application of biosensor.

Temperature measurement in the food safety is a Critical Control Point (CCP). Thermocouples are widely used because of its reliability and low cost (Zell et al., 2009) but it generate large degree of uncertainty and local limited information (Wold, 2016, Guérin et al., 2007). These features have been opening windows for new methods.

Combining temperature sensor or indicator (TTI) with an Radio Frequency Identification (RFID) tag can be the best choice for products in the chilling chain (Wan and Knoll, 2016). It confers the wireless characteristics representing the next generation on monitoring temperature. For this purpose, RFID technology is recognized as a new generation of smart RFID tag for intelligent food packaging and notorious advantage by reduction and simplification in wiring and harness (Badia-Melis et al., 2014, Kim et al., 2016b). The researches combining TTI-RFID in the cold chain and examples of wireless sensor (Dwivedi and Ramaswamy, 2010) reinforce the potential use of RFID in processing steps in the food industry.

Our research group has been studying the electrical properties of biopolymers to investigate how these properties depend on the temperature and humidity (Bibi et al., 2016). Proteins are good candidates because of their sustainability coming from renewable resources and from by-products. Another advantage is the potential use for both temperature monitoring and/or humidity and food quality makers (as biosensor).

Combination of biology and electronics is a promise for on-line measurements of important process parameters and microbial detection (Ramaswamy et al., 2007). Our proposal is the use of biopolymers as innovative sensors of temperature. The objectives of this project were: part 1: to prepare a review to get stat of art of the sensor of temperature; part 2: to select the biomaterial based on the electric capacitance; part 3: (1) to study the influence of the layer thickness on the electrical capacitance sensitivity, (2) to evaluate its application under meat cooking protocol, and (3) to evaluate its stability for continuous use of the same sensor; part 4: study the RFID performance of gelatin as sensor of temperature.

2. Material and Methods

The electrical properties were studied under different temperatures (20°C up to 80°C) and humidity (90% RH).

Part 1: Literature review of temperature sensor.

Part 2: two techniques were used: electrical capacitance of sample coated onto interdigital electrodes (IDC system) and real permittivity and loss factor of self-supported sample (volumetric capacitance) to evaluate the biopolymers: gelatin, sodium caseinate and soybean isolated protein. Specific step 2.4.

Part 3: It was used gelatin (selected in the step 1) coated onto interdigital electrodes (IDC system). Specifics steps 2.5 and 2.6.

Part 4: it was used gelatin coated onto RFID tag. Specific step 2.9.

2.2. Preparing the solution

10% w/v of soybean protein isolated, sodium caseinate and gelatin prepared as shown by (Arfa et al., 2007, Helal et al., 2012, Fakhoury et al., 2012). The bubbles were removed under vacuum.

2.3. Thickness

The average thickness of the samples was measured by a hand-held digital micrometer (0.001 mm). For the thickness at RFID tag it was used a profilometer.

2.4. Thermogravimetric Analysis (TGA)

Thermogravimetric analyses (TG/DTG) were run in a TGA, model Pyris 1, Perkin-Elmer. The carrier gas was nitrogen at a flow rate of 30 mL/min. The temperature range was 20°C to 80°C, at a heating rate of 10°C/min. The analyses were made in duplicate.

2.5. Differential scanning calorimetry (DSC)

The thermal analysis of the gelatin was carried out in a DSC from Perkin-Elmer, model Diamond, with an external refrigerating device (Intercooler II) and nitrogen as a purge gas system, with a flow rate of 20 $mL \cdot min^{-1}$. The temperature range was 25°C–170°C, at a heating rate of 10°C/min. The analyses were made in triplicate.

2.6. Scanning Electron Microscopy (SEM)

The SEM analysis was carried out in a FEI Quanta 200 FEG. It is equipped with **X-Max**^{50mm2} (Silicon Drift Detector), manufactured by Oxford Instruments. The sample was composed by gelatin coated IDC system on the SEM stubs. The analysis on nitrogen, carbon and copper were made by the software of the equipment.

2.7. Samples Coated onto Interdigital Electrodes

2.7.1. Preparing Samples

The solutions were coated onto the surface of the interdigital electrodes with circuit reference of 1 GHz (Cirly, France), by the film applicator Coatmaster 510 (Erichsen, Germany), followed by a drying step at room temperature and relative humidity (around 25°C and 50%, respectively). A blank uncoated electrode was also used as a reference.

The method of immobilization of the proteins onto the surface of the electrode was of the adsorption.

2.7.2. Determination of the electrical capacitance

The electrical capacitance was determined according to Gervogian model (Wang et al., 2003). It was used Impedance Analyser HP 4191A RF, at a frequency range of 300 to 900 MHz, that was linked to interdigital electrodes by a coaxial cable semi-rigid SMA (Amphenol Connex, France) and to the connector coaxial SMA 500HM Solder SMA (Amphenol Connex, France). The temperature and humidity were controlled by a climatic chamber (Secasi Technologies, France). For the measurements, the sample was conditioned at 20% and the temperatures varied (20°C, 50°C and 80°C). The process was repeated at 55% and 90% RH. The software used to record the results was LabView (National Instruments).

2.8. Self-supporting Samples

2.8.1. Preparing the Samples

The solutions were poured in a plastic container to form a wet film, with a thickness of approximately 0.8 cm. After, they were dried at room temperature. They were cut with a borer in order to get samples with 2 cm of diameter (before being completely dried). A teflon film was also used as a reference.

2.8.2. Humidity conditioning

The humidity dependence was evaluated at two different water activities (a_w). The samples were conditioned in desiccators with saturated solutions of potassium carbonate (111 g/100 mL) and potassium nitrate (47 g/100 mL) salts. The a_w was taken daily in triplicate (FA-st/1, GBX) up to stability. For gelatin, sodium caseinate and soybean isolated protein, the stabilized a_w values were 0.82, 0.87, 0.85 for nitrate carbonate and 0.40, 0.38 and 0.43 for potassium carbonate, respectively.

2.8.3. Determining the Volumetric Capacitance

The response variables were dielectric constant and the loss factor, according to the equation:

$$C = \frac{\varepsilon_r \varepsilon_0 A}{d}$$

where C is the capacitance, ε_r is the relative permittivity, ε_0 is the vacuum permittivity (8.85×10^{-12} F/m), *A* is the electrode surface, and *d* is the distance between the two electrodes (sample thickness). The Impedance Material Analyser HP 4291B was used from 1 MHz to 1.8 GHz. Five measurements were performed for each sample.

2.9. RFID performance

The performance of RFID tags was evaluated by Voyantic (Finland) directional coupler 700-1200 MHz. The gelatin sensor-enable RFID tags were positioned onto an antenna inside of climatic chamber (Espec, Japan) to control both humidity and temperature. The link of the support and the Voyantic was made by the RF cable and the measurements were recorded with the Tagformance Measurement Software.

After stabilization of humidity (90% RH) and each temperature, the measures were made under frequency band of 700 MHz up to 1200 MHz. The temperatures used were 20°C, 40°C, 60°C and 80°C. After arriving 80°C is was taken measures for the descending temperature: 60°C, 40°C and 20°C.

Three layouts were tested and compared with an uncoated tag: the gelatin film was coated onto (a) layout 1- all tag except chip area (b) layout 2- chip area, and (c) layout 3- internal loop.

2.10. Statistical Analyses

For all statistical analyses, it was used 5% as a significant level and the Statistica software, for Windows, version 12.0 (Tulsa, USA). All data are presented as average values ± standard deviations. For the part 1 it was used a 3^3 factorial design with three central points (annexes 1 and 2).

3. Results and Discussion

Part 1: Literature review

The temperature is monitored inside the food industry mainly by thermocouple that is based on spot checks on a small number of products generating large degree of uncertainty and local limited information. It can lead to over-cook much of the food to ensure that everything has reached the critical core temperature (Wold, 2016, Guérin et al., 2007). These features have evoked news methods for temperature monitoring focusing mainly non-contact (non-invasive) temperature measurements (Knoerzer et al., 2009, Eder et al., 2009, Wan and Knoll, 2016) and also wireless.

The performance of wireless sensor was analysed relative to conventional thermocouples sensors for temperature monitoring during canned food sterilization. Statistically, the two sensors did not differ ($P > 0.05$) with respect to gathered temperature data. For rotary retorts, they offer excellent advantages, and in continuous flow rotary systems (Dwivedi and Ramaswamy, 2010).

To monitoring temperature at post processing, the advanced technique is time-temperature indicator (TTI). In particular, if the TTI is incorporated with RFID tag will be a new generation of smart RFID tag for intelligent food packaging (Badia-Melis et al., 2014, Kim et al., 2016b).

There is no use of RFID technology to monitor temperature during processing steps. The examples of wireless sensor and TTI-RFID are the bases for the use of an innovative use of sensor-enable RFID for this purpose.

Part 2: Selecting of sensing biomaterial

According to the Thermogravimetric analysis (TG), the onset degradation temperatures, taken just after the first stage, were 290.4 ± 11.9°C, 315.7 ± 3.6°C and 286.1 ± 0.6°C for sodium caseinate, gelatin and soybean isolated protein, respectively. Thus there is no evidence of degradation at temperature normally used for meat cooking (80°C).

The electrical capacitance of all biopolymers was frequency dependent; it increases as frequency increases. This behavior was more intense at higher humidity (90% RH) what agrees with literature (Ryynänen, 1995, Zhu et al., 2010).

Gelatin has shown higher capacitance; for soybean isolated protein and sodium caseinate, the values were similar. Higher molecular weight of gelatin (300 kDa) (Figueiró et al., 2004) comparing to 20.2 to 81.4 kDa (Martins, 2005), for soybean isolated protein, and 23 kDa (Gubbins et al., 2003) for sodium caseinate can explain its better performance. This parameter can change the electrical behavior (Kolesov, 1968).

However, the molecular size of gelatin (length of 300 nm and diameter of 1.5 nm (Figueiró et al., 2004) is much smaller than the wavelength at UHF (1 m-10 cm (Sanghera and

Thornton, 2007), leading to a dependence of electrical properties only on the shape (Ryynänen, 1995).

Difference in molecular shapes leads to different surface areas, interfacial areas and interfacial polarization; consequently, the dielectric properties change (Dang et al., 2012). The main characteristic of gelatin is the triple-helical domains (Rest et al., 1993), that is stabilized by both interchain hydrogen bonds and ‘structural’ water molecules (Sarti and Scandola, 1995).The ability of its unfolded peptide chains to trap a large amount of water molecules allows to utilize their intrinsic dielectric properties (Sanwlani et al., 2011). Even though the large number of C=O…H-N hydrogen bonds limits the mobility of the polarized groups, they are on a large number, which is beneficial for the polarization (Ning et al., 2015).

The sensitivity to environmental conditions, such as temperature and relative humidity, is reported as a very important restriction factor concerning the films because they are hydrophilic materials and thus very susceptible to water (Gennadios, 1993). The sensitivity to water vapor is reported as the largest challenge for their practical applications (Potyrailo et al., 2011). However, in our work, this sensitivity was suitable as it has changed the electrical properties indicating the humidity and temperature dependence.

Both temperature and humidity influence the capacitance (Foucaran et al., 2000). But only at 90% RH, because of water polarization, this influence was significant to distinguish the temperatures. This is a desired condition, once the humidity used during the meat cooking is around 90-95%. Regarding to 90% RH, the Fisher’s test shows that, for SIP, GEL and SCA, there was a statistical significant difference ($p < 0.05$) only between 20^{o}C and 80^{o}C. However, only gelatin was able to have a statistical significant difference for capacitance between 20^{o}C and 50^{o}C, a temperature range common in food industries.

Part 3: Biomaterial evaluation

One material can be ionized and become a conductor as no dielectric material is a perfect insulator. This was observed at samples of gelatin sensor at high thickness (61 µm and 125 µm). This tendency can be related to the dielectric strength according to the theories of Artbauer and electro-thermal breakdown (Li et al., 2015).

Both theories are based on the influence of temperature, variable that was assumed to explain the final stage of a breakdown process (Ho and Jow, 2012). At 868 MHz, there was a conductor effect between 60°C and 80°C that has finished after returning to 60°C.

The Artbauer theory was confirmed in DSC measurements of polypropylene foils that have revealed strong correlation between structural phase transitions at the same temperature regions as it shows discontinuities in the breakdown strength (Schneuwly et al., 1998). The same was observed with the electrical properties of gelatin, once between 60°C and 80°C the curve of capacitance dropped and it is quite the same band of temperature where the T_g started and finished, whose extrapolated value was 77.84 $\pm$ 0.13°C.

Both theories mentioned and explained the breakdown temperature dependence that appeared only for thicker samples (61 μm and 125 μm) showing that thickness is also a variable that influences the electro-thermal breakdown (Schneider et al., 2015). Thus, these results point out to the necessity of a good balance between thickness and frequency to the adequate use of gelatin sensor. Based on our early studies with real permittivity of gelatin, 600 MHz was the last frequency before reaching the resonant frequency and it was also the frequency observed just before starting this phenomenon with sample at 125 μm; this thickness was chosen to continue the studies, comparing to 38 μm, once higher sensitivity was obtained relating to 61 μm.

The hysteresis for 38 μm and 125 μm were evaluated. Both curves (40-80°C and 80-40°C) for 38 μm were quite linear and, for 125 μm, the linearity of the curve was adequate for the rising temperature, but it has changed for the descending one. The maximum hysteresis correspond to a 6% of capacitance at 40°C (125 μm), but for all other points, it was below 2%, exhibiting a narrow hysteresis loop; result supported by literature (Zhu et al., 2010). In our previous tests, it was observed the tendency of stabilization in different levels for the same temperature (rising and descending), mainly for samples thicker than 50 μm.

For the relation temperature versus capacitance, the sample with 125 μm presented a curve with a higher slope, indicating a higher sensitivity that was calculated. The sensitivity for the sample with 125 μm was 0.14 pF/°C, while for the sample 38 μm it was 0.045 pF/°C, being more than 3 times lower. It showed that higher thickness leads to higher effectiveness to distinguish the variation of temperature.

The higher sensitivity of thicker samples was determinant in the test simulating the temperature and humidity used during meat cooking. The gelatin sensors (38 μm at 868 MHz and 125 μm at 600 MHz) were tested following the steps of meat cooking (Fig. 5). It is clearly seen that the higher thickness (125 μm) led to a more distinguishable results mainly in the cooling steps, which are important for the effective food safety. Considering ready-to-eat products, such as ham, sausages, it is postulated a cooling step, from 54.4 to 26.7°C, no longer than 1.5 h and from 26.7 to 4.4 °C, no longer than 5 h (USDA/FSIS, 2001), essential to reduce the activity of pathogenic microorganisms (Mohamed, 2008). Both samples were able to show different electrical capacitances; however, with 125 μm, the system is more robust.

The repeatability of capacitance reading of the same gelatin sensor was investigated by using it thrice at three different temperatures (40ºC, 60ºC and 80ºC), after storage at room temperature (around 25ºC) and humidity equals to 60%, approximately. The capacitance value obtained at the first measurement was considered as the reference. In general, the capacitance reduction was around 30% and 50% for the second and third times, respectively. The capacitance obtained at each time and temperature was the result of the average of three measurements (repetitions). The coefficient of variation was lower than 3%, showing a data robustness. The explanations for the reduction comparing to the reference was due to loss of material. Indeed, the most important indicator that inhibits a continuous use of the sensor is not related to electrical measurement, but to the reduction of sensitivity. After storage at low humidity, for the third time, it was 0.019 pF/°C, more than two times lower than the first time (0.045 pF/°C).

Part 4: Coupling of sensing biomaterial with RFID chips: sensing RFID tag

The RFID system can be operated in several frequency bands, but the most used is the Ultra High Frequency (UHF), specifically the frequencies managed by regulations of individual countries:868 MHz (Europe) and 915 MHz (United States) (Sanghera, 2007). At UHF, there are many advantages, such as: transfer data faster than low and high-frequencies (Ruiz-Garcia and Lunadei, 2011), longer communication distance, higher data rates, as well as smaller antenna size in RFID systems (Sun et al., 2010). This lack of standardized frequency is hampering the implementation of RFID technology for different applications (Sanghera, 2007). It is reported by the literature that as 915 MHz and 868 MHz are close frequencies, the propagation characteristics and conclusions can be also extended to each other (Angle et al., 2014). This approach is not totally applicable because there was a significant difference for the layouts 1 (and 2 ($p < 0.05$) between the aforementioned frequencies, indicating different behaviour.

The Fisher's test, considering 5% of significance level, showed that temperature, layout, frequency and their interaction effects influenced significantly the radio frequency answer. Comparing the results of the three layouts with the reference layout (without gelatin film), it is clear there is an influence of the gelatin on the sensor-RFID response. However, the better performance of the layout 1 (coverage of whole antenna) in terms of absolute value of relative variation was outstanding confirming the importance of the whole coverage of the antenna as the layout suitable for monitoring the temperature.

The TRR is a result of a given temperature and a correlation between them may be stablished; it is desirable that the TRR value for rising temperature would be the same for descending temperature, implying then no hysteresis. In layout 1 at 915 MHz, this condition was fulfilled at a critical temperature zone that is necessary for the effective control of pathogens such as *Clostridium perfringens* (60°C up to 80°C and 80°C up to 20°C). Even though in layouts 2 and 3 at 915 MHz and 960 MHz the absence of hysteresis was observed, there was no significant difference among the different temperatures (20°C, 40°C, 60°C and 80°C); thus, they are not suitable for monitoring the temperature at 915 MHz and 960 MHz.

Besides the behaviour on the hysteresis, the sensitivity at 915 MHz was also remarkable comparing to the others frequencies (868 MHz and 960 MHz); it may be seen by the inclination of the curves. The hysteresis error was 28% and 31% for 868 MHz and 960 MHz, respectively; these values are around 3 times comparing to 915 MHz whose value was 10% at 40°C that is inside the acceptable band of variation. Further, the sensitivity was influenced by the temperature band and also the rising (up) and decreasing temperature (down) and by this variable it can be seen also the outstanding results at 915 MHz.

The frequencies normally used in UHF RFID system operate with reduced readability near loads of perishable products with high-water content. Water absorbs radio frequency energy, decreasing the read range (Amador and Emond, 2010). Taking as reference the normal value of read range for passive tag at 860-960 MHz, that is below 10 m (Plos and Maierhofer, 2013), it can be seen that in all layouts the TRR values were inside this limit showing trustworthiness. However, the influence of the temperature is observed at 80°C once at this value the TRR was above 10 m.

The influence of water may be considered as a key noise parameter (KNP), as it reduces the read range. The knowledge of KNP is mandatory in systems based on electromagnetic waves, such as RFID. In our previous studies (to be published), essays were carried out under humidity of 40% and 90% RH and the influence of water on the TRR changes markedly in function of humidity and frequency. For 840 MHz, the TRR variation was around 90% up to 130% for 20°C and 60°C, respectively, and for 868 MHz the variation was around 225%. However, considering 80°C for both frequencies, the variation of TRR was around 90% and 100% (840 MHz and 868 MHz, respectively). This lower TRR variation compared to 60°C may be related to an influence of the gelatin glass transition (Tg) (Boltshauser et al., 1991, Story et al., 1995). Thus, beside water influence, there is also an influence of Tg on the TRR. Herein, both water and Tg did not preclude the sensitivity in all three layouts, showing the robustness of this new sensor to overcome these KNPs.

Based on 868 MHz, 915 MHz and 960 MHz, it may conclude that layout 1, compared to layout 2, was superior once there was a significant difference in TRR values at the critical temperature zone: heating (60°C up to 80°C) and cooling (80°C up to 20°C) for all frequencies. Thus, it confers flexibility to attend the different regulations of the countries regarding to which frequency, 868 MHz, 915 MHz or 960 MHz, is adopted.

For the regions where 868 MHz is used, layout 2 may be adopted but, at this frequency, layout 1 is more suitable to be used as it permits to distinguish better the difference of TRR values among the temperatures. It is not possible to use layout 3 for all frequencies (868 MHz, 915 MHz and 960 MHz), because there was not a significant difference in TRR values among the temperatures.

These results show that the way the gelatin was coated onto the tag (Fig. 1) clearly influences the TRR value. Based on layouts 2 and 3, it may be inferred that the coverage of the chip area in the first one was the explanation for better results. The coverage of antenna in layout 1 (without the chip area being covered as well) was the key feature for the temperature sensor. As the antenna transmits information, it is reasonable to restrict a contact with the sensing material (gelatin).

5. Conclusions

Two different techniques, sample self-supported and coated onto interdigital electrode, were used and both have shown the influence of water polarization. Gelatin, because of its molecular shape and chemical characteristics, was the most sensitive sensor.

For the sample with higher thickness (125 µm), the temperature induced the electro-thermal breakdown (around the temperature range of 60°C up to 80°C) limiting the use at 868 MHz. But a balance between higher thickness and frequency permits the use of the gelatin sensor with higher sensitivity. The experiments with sample with 125 µm were carried out at 600 MHz, this combination in comparison with sample with 38 µm at 868 MHz resulted in a higher sensitivity and in a better condition to distinguish the different temperatures normally used in the meat cooking. The gelatin sensor may be used several times under the same and continuous experimental conditions (90% RH and up to 80°C) without variation in the capacitance but reuse of the same gelatin sensor several times is not recommendable because it reduces the sensitivity as a result of mass loss after each use. The gelatin sensor-enable RFID tag had good performance for monitoring the temperature. For 868 MHz, 915 MHz and 960 MHz, the layout 1 was suitable because it was able to deliver different results ($p<0.05$) for all frequencies and it was the only at 915 MHz, that the condition of no hysteresis was fulfilled at a critical temperature zone (60°C up to 80°C and 80°C up to 20°C). We obtain better results for 915 MHz with an error hysteresis of 10% and a sensitivity of twenty twice important than the others frequencies (868 MHz and 960MHz). Moreover, the layout 1 at 915 MHz, points the potential use of this new sensor for heating and cooling steps during meat cooking.

Part I: Literature review

SENSOR-ENABLE RFID TAGS: FEASIBLE NEXT GENERATION FOR MONITORING TEMPERATURE IN FOOD INDUSTRY

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Abstract

Temperature is one of the most important variables in food industries and its effective control impacts on obtaining products under microbiological control standards. Thus, a review about temperature monitoring in the food industry, essential for food safety, is presented. Some methods, like WSN (Wireless Sensor Networks) have been proposed to substitute thermocouple because of its limitations, such as incompatibility with automatic loading systems. RFID, coupled with time-temperature indicator (TTI-RFID), has been applied successfully for monitoring temperature in the cold chain. These technologies represent desirable characteristics for new methods, for example non-contact, non-invasive and wireless thermometers. The focus of this review was to introduce RFID, based on examples of wireless sensor and (TTI-RFID), as a future trend for an innovative tool to monitor the temperature in processing steps. This new feature may also open possibility to integrate the production channel and both processing and post processing steps.

Keywords: RFID; Temperature sensor; Monitoring temperature; Food safety; Food quality control.

1. Introduction

Thermal treatment is the most used method focusing destruction of pathogenic microorganisms. The temperature control is one of the major environmental factors to address the food safety, especially for ready-to-eat products, whose consumption may be done just after finishing the cooking step (Wold, 2016, Pham, 2014). For food safety purpose, monitoring temperature of perishable food must be effectively made inside the industries and after packaging (post processing). This approach intends to share responsibilities with different stakeholders aiming at delivering appropriate processed products to consumers.

For food processing, the temperature is usually monitored by thermocouples that are widely used because of their reliability, of their price and their robust method of measuring temperature over a wide range (Zell et al., 2009, Gillespie et al., 2016). But thermocouples are based on spot checks (contact or invasive sensor) of a small number of products generating a high degree of uncertainty and local limited information. These aspects can lead to overcook in order to ensure that the target temperature was applied to all products (Wold, 2016, Guérin et al., 2007).

These features have evoked news methods for temperature monitoring focusing mainly in non-contact (non-invasive) temperature measurements (Knoerzer et al., 2009, Eder et al., 2009, Wan and Knoll, 2016). A desirable method may register the temperature in all food products (Wold, 2016). However, aspects, such as cost and complexity, are delaying their use. A potential method is the wireless sensor that meets quite the same characteristics of thermocouple and adds the advantage of wireless feature (Dwivedi and Ramaswamy, 2010).

After process steps, it is current to store food in climate chambers whose temperature is controlled by simple thermometers. This context fails as it does not permit to identify regions of the chamber with inefficient cooling coming from technical problems or arrangement of products. This technique does not also allow to register any temperature oscillation.

A new concept lies under the use of time-temperature indicator (TTI) that exhibits an easily measurable and inexpensive way to monitor and communicate the temperature measurement. It integrates the temperature history of the product displaying a best-before date label and efficient shelf-life management (Wan and Knoll, 2016, Taoukis, 2010, Kim et al., 2016b).

Nowadays, researches are made in order to couple TTI with RFID conferring wireless characteristics that represent the next generation on monitoring temperature. For this purpose, RFID technology is recognized as a potential tool of smart tag, inside intelligent packaging concept, with remarkable advantage of reduction and simplification in wiring (Badia-Melis et al., 2014, Kim et al., 2016b).

Perishable foods need proper temperature-controlled environments during the production, storage, transportation and sales processes to ensure food quality and to reduce food losses (Aung and Chang, 2014). The aim of this review was to present the main methods of temperature monitoring and a focus was made on the RFID technology as a potential tool to integrate food processing and post processing steps.

2. Monitoring temperature inside the industry

In food industries, temperature monitoring is made mainly by thermocouples (TC). It provides a simple, easy, inexpensive and robust method of measuring temperature over a wide range, what explains its spread use (Gillespie et al., 2016, Wold, 2016).

In general, the accuracy of thermocouple is limited to 0.5°C. Major error sources include connection wires, cold junction uncertainties, amplifier error, and sensor placement (Williams, 2011). Other disadvantages are:

- Produce spot check, generating a high degree of uncertainty, lack of sensitivity, local limited information and measurement inaccuracy (Wold, 2016, Kasper et al., 2013).
- There is an impossibility of use in microwave heating, method that uses electromagnetic waves, proposed as an alternative to traditional heating. The metallic wires disturb the waves, which may destroy the thermocouple and damage the product (Knoerzer et al., 2009, Sung and Kang, 2014).
- Chemical erosion is related to thermocouple, leading to sterility concerns (Kasper et al., 2013). This aspect is relevant for non-destructive samples.
- TCs are not compatible with automatic loading systems (Kasper et al., 2013).

The goal of new methods must consider engineering requirements, provide local unlimited information and be on-line. Besides these technical challenges, they must overcome inherent characteristics of the food industry such as opaque products and presence of metal and deliver 3D temperature fields (Guérin et al., 2007). Moreover, they should be also based on non-contact (need for stringent sterile conditions) and/or non-invasive thermometers (Nott and Hall, 1999, Cuibus, 2013).

The main disadvantage of practically all methods is to monitor the temperature on the surface of the products not in the cold point (Lee and Yook, 2014). Correlation between external and internal temperatures should be carried out in order to validate the method (Ibarra et al., 2000).

Several techniques intended to substitute thermocouples are presented next in a non-exhaustive list that includes methods that may not be feasible but are still better. It is remarkable that all of them are used in other areas, such as medicine, for other purposes besides monitoring temperature, which implies flexibility of use.

2.1. Optical Fiber Sensor

Optical Fiber Sensors use the properties of light passing through a glass fiber to measure the temperature besides other variables. The advantages reported related to this method are high sensitivity, light weight, small size, large bandwidth, immunity to electromagnetic interference, and ease in signal light transmission (Lee et al., 2013).

Immunity to electromagnetic interference may postulate this method for microwave heating, even though there is a challenge to obtain good spatial resolution. Comparing to thermocouples, this method is delicate and expensive (Knoerzer et al., 2009).

This method was used to control the centre and circumference temperatures during defrosting of tuna by radiofrequency (Llave et al., 2015). It was also proposed as new device to monitor the temperature during lyophilisation. Comparing to data obtained with conventional thermocouples, the results have showed significantly higher sensitivity, faster response and better resolution (Kasper et al., 2013).

2.2. Thermochromic Liquid Crystals

Thermochromic Liquid Crystals (TLCs) are temperature indicators that modify incident white light and display different colour whose wavelength is proportional to temperature (Stasiek et al., 2006, Balasubramaniam and Sastry, 1995). This method has been widely used by researches groups to map the surface temperature and spatial distributions. The fast response and non-invasive characteristics turn TLCs useful where other conventional temperature sensors cannot be applied (Abdullah et al., 2010, Balasubramaniam and Sastry, 1995).

The quantitative use is possible after firstly determine the correlation colour–temperature and calibrate it with a thermocouple at a known temperature range. Some disadvantages of this method are the reversible colour changes upon cooling avoiding the thermal history and the necessity of transparent media between camera and surface allowing correct identification of the colour (Abdullah et al., 2010, Balasubramaniam and Sastry, 1995). These setbacks limit the effective use of this method in food industries.

2.3. Infrared Thermography

Infrared Thermography (IRT) has become a matured and widely accepted tool to monitor the temperature based on real time, non-contact and reasonably accurate readout features (Bagavathiappan et al., 2013, Knoerzer et al., 2009). The fast determination of the temperature is the major reason for its growing demand in various fields (Vadivambal and Jayas, 2011). However, it has been used in a small part of the food industry because of its high price and the difficulty of use.

Examples of successful use were the heating control of eggs and freezing process of potatoes (Cuibus, 2013). Also, many food-processing operations are accompanied by airborne particulates, such as smoke or water vapor, that disperse the infrared radiation and reduce the accuracy of readings (Stephan et al., 2007). Because of the metallic components, IRT has its use avoided in microwave oven that uses electromagnetic fields (Knoerzer et al., 2009).

2.4. Microwave Radiometry

Microwave Radiometry (MR) provides precise measurement of the temperature based on the principle that radiation intensity is proportional to a given temperature (Toutouzas et al., 2011).

The physical principles are similar to those of infrared thermometry, except that microwaves are not absorbed or scattered significantly by airborne particulate clouds that disable infrared sensors that use much shorter wavelengths. Depending on the wavelength chosen, microwave temperature measurements can provide data of interior as well as surface temperatures (Stephan et al., 2007).

The possibility to measure is because of the microwave frequency has higher penetration depth comparing to infrared frequency range. It is reported penetration of several millimetres below the surface and centimetres (Knoerzer et al., 2009, Nott and Hall, 1999). This feature does not achieve the cold point in thick products, but its potential as temperature sensor was shown with hamburger with thickness of 0.8mm (Stephan et al., 2007).

2.5. Magnetic Resonance Imaging

Magnetic Resonance Imaging, among other techniques for measuring temperatures in electromagnetic fields, is pointed as more suitable, but its cost exceeds at least one order of magnitude comparing to the other potential substitutes of thermocouple. However, for premium products, with higher added value, this higher cost may be compensated (Knoerzer et al., 2009). It is an efficient method that permits good spatial resolutions for surface temperatures and also for temperature distributions throughout the product.

Magnetic Resonance Imaging is the physical process that the nucleus, whose magnetic moment is not zero, resonantly absorbs radiation of a certain frequency under external magnetic field. Despite being an efficient method, its use is mainly made in medical area and in food industries focusing on quality control by images (Chen et al., 2013).

2.6. Ultrasonic method

Ultrasonic methods are simple, accurate, rapid, and non-destructive(Kiełczyński et al., 2014). It is based on the ultrasound velocity (C) that in any medium is generally a function of inner temperature: C = f(T) (Richardson and Povey, 1990)

Its feasibility was investigated by several authors in the past (Richardson and Povey, 1990). Meanwhile, its use is foreseen by monitoring temperature, under food industry context. Almost none research was developed with this purpose but for other applications such as investigation of liquids (Kiełczyński et al., 2014) and estimation of chemical composition (Nowak et al., 2015). Because of its complexity, this method is much more expensive compared to thermocouples, but it could be relatively cheap and suitable for mobile applications (Nowak et al., 2015).

2.7. Radiation Thermometry

Radiation thermometry is a non-invasive technique capable to deliver temperature measurements with the lowest uncertainties. It has several unique advantages, such as the ability to reliably follow rapid temperature changes. In some cases, it can measure small objects or map the temperature distribution with a spatial resolution of a few micrometres (Yoon and Eppeldauer, 2009).

Commercial radiation thermometers have their use foreseen to control surface temperature during heating or freezing, avoiding the use of a contact thermometer to each single product. However, this method needs a free surface to be applied, restricting its use in closed containers, for instance (Eder et al., 2009).

When a radiation thermometer is used to measure a surface temperature, two issues arise. Firstly the unknown emissivity of the surface (which affects the emitted radiation from the target) and the second is the influence of the emission from and absorption by the environment (which can significantly influence the radiation reaching the detector) (Zhang and Machin, 2009).

2.8. Wireless sensor

During the last years, wireless technologies have been under rapid development (Wang and Li, 2013). This technology is composed of radio frequency transceivers, sensors, microcontrollers and power sources. Deployment of wireless sensors in the agriculture and food industries is still rare but with great potential of use. Their obvious advantage is a significant reduction and simplification in wiring harnesses and connectors reducing maintenance complexity and costs (Dwivedi and Ramaswamy, 2010, Wang and Li, 2013).

Wireless sensor was applied successfully for monitoring cold chain in the kitchen, vehicle of transport and retail store segments. The management was made by an alarm that rang when temperature records were missing or the package temperature fell out of the acceptable range (up to 7°C during refrigeration) including re-cooking if temperature dropped below 80°C (Shih and Wang, 2016).

The performance of wireless sensor was analysed relative to conventional thermocouple sensors for temperature monitoring during canned food sterilization. There was not statistic difference between the two sensors (P>0.05). For rotary retorts and continuous flow rotary systems, they offer excellent advantages. However, they are relatively expensive and available only from selected suppliers (Dwivedi and Ramaswamy, 2010).

The setbacks related to this technology in the past, such as lack of standardization (Wang and Li, 2013), are still ongoing challenges for researches. More studies should be made in order to address reliability and to reduce the risky for process control (Dwivedi and Ramaswamy, 2010). Wireless sensor is a potential tool that can substitute thermocouples and can be also the link for monitoring a temperature of the same product in different steps including cold chain and production, such as heating and cooling. The advantage for producers is a more efficient application of the Good Manufacturing Practices principles relate to documentation and registration.

3. Post processing temperature monitoring

The communication of conventional packaging is made only by the label and this was the drive force for the development of an intelligent packaging (IP) contributing to a better shelf life control and providing information about the quality and safety status of food (Taoukis and Tsironi, 2016). This approach permits the early warning that is desirable concerning of food safety.

Following the concept of IP, it takes place the “smart labels” that are attached on food packages exhibiting an easily measurable response that indicates the product quality level (Kim et al., 2016b). There are two categories of smart labels: freshness indicators, that provide direct product quality information, and time–temperature integrators or indicator (TTIs).

TTI exhibits an easily measurable, inexpensive and cost-efficient response that permits to get the temperature history throughout distribution resulting in a realistic control of the chill chain and reduction of waste. They are usually based on various processes, such as the diffusion of colourful solution, colour-changing polymerization, enzymatic reactions, and photochromic reactions displaying a best-before date label and efficient shelf-life management (Wan and Knoll, 2016, Taoukis, 2010, Kim et al., 2016b).

The viability of TTI use was shown and the tendency is to carry more researches in order to establish pattern permitting broad application. TTI based on biofuel cell could successfully predict milk quality changes (Kim et al., 2016b). Study indicated that the *Vibrio vulnificus* and *Vibrio parahaemolyticus* (Tis) may be an effective and cost-effective tool for validating improved handling and cooling procedures and for monitoring oyster transport (Tsironi et al.). Acting as smart label, microbial TTIs may accumulate the temperature history and indicate the food quality decline (Zhang et al., 2016). Isopropyl palmitate diffusion-based TTI system was successfully applied for monitoring microbial quality of non-pasteurized angelica juice based on temperature abuse (Kim et al., 2016a).

3.1. TTI and RFID

Food distribution is complex and huge; the introduction of information technology concept such as radio frequency identification (RFID tag) is desirable to better monitoring of the distribution channel. As TTI has an electrical sensor function, it could be linked with RFID tag (Wan and Knoll, 2016). Recently, RFID technology is presented as an appropriate tool to measure temperature in both ambient context and food packaging system (Trebar et al., 2015).

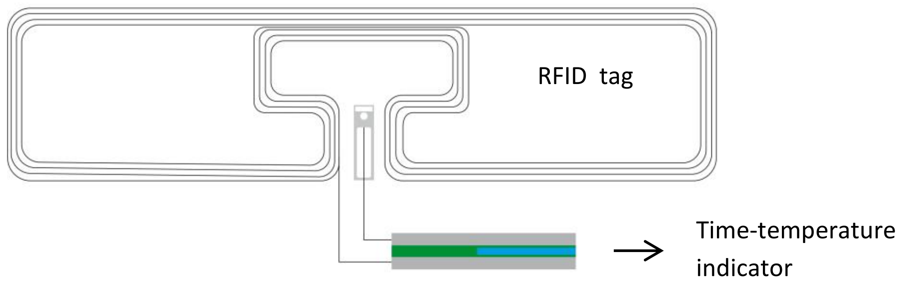

Fig. I-1. Scheme of time-temperature indicator coupled with RFID tag.

Incorporating TTI with RFID tag (Fig. I-1), represent a new generation of smart RFID tag with additional advantage by reduction and simplification in wiring (Badia-Melis et al., 2014, Kim et al., 2016b). A combination of both technologies (TTI-RFID) can offer double protection for perishable food (Wan and Knoll, 2016). RFID tag provides a unique advantage to monitor the supply chain in real time by the simple use of a RFID reader in strategic points (Lorite et al., 2017).

3.2. Radio Frequency Identification (RFID)

Radio frequency identification (RFID) technology is an emerging and significantly advantageous technology for food industries (Tanner, 2016). RFID allows the use of an specific tag for each product that can be monitored at any point of the entire supply chain reducing food loss (Wang and Li, 2013).

RFID is reported as an Automatic Identification providing electronic information under intelligent packaging (IP) concept. It is not classified as sensor or indicator once it does not provide qualitative or quantitative information (Kerry et al., 2006, Vanderroost et al., 2014). However, categorize RFID as IP is not unanimously accepted, because they are not responsive to and informative about the kinetic changes related to quality of food product (Yucel, 2016).

While there is a discussion about if RFID is IP or not, the potential use as a tool to integrate the production chain is well demonstrated for TTI-RFID. Coupling the RFID tag with a sensor/indicator could combine the advantages of both technologies. The literature has shown this feasibility and points its potential to be applied in a low-cost, large volume manufacturing and for items that require stringent temperature management (Nakayama et al., 2016, Myny et al., 2010). RFID can enhance the performance of IP when used alongside a sensor by providing location-specific information (Yucel, 2016).

Coupling RFID with indicators represents the vanguard of technologies to be introduced in IP system and the challenge lies on coupling one or more sensors in the RFID tags and integration in packaging materials. In terms of application of this technology, the future scenario points to provide information relating to the integrity of the package, quality status and environmental variables such as temperature and volatile compounds (Vanderroost et al., 2014).

4. Feasibility sensor-enable RFID as next generation

Technologies, such as sensors, Radio Frequency Identification (RFID) and wireless networks (WSN),are key components to ensure visibility of each product throughout their life cycle (Aung and Chang, 2014). Assembling all of them can be the best answers for the self-enabled RFID. Wireless sensor network (WSN) is used to collect and transmit information about the environment. RFID transmit information of an object identified by unique serial number. The complementary of both lies on their different objectives that increases the effectiveness of the monitoring (Jain, 2010). This integration provides more product information in addition to identification (Wang and Li, 2013, Ruiz-Garcia et al., 2009). Both wireless sensor and RFID are technologies that face with the targets of the new methods for monitoring temperature, such as to be on line and wireless application. RFID is a new frontier in terms of wireless technology.

Monitoring and tracking the temperature in the cold chain are the main objective of RFID application in food industries and for these purposes the results have shown viability (Badia-Melis et al., 2014, Jedermann et al., 2009, Trebar et al., 2015, de las Morenas et al., 2014). The temperature was monitored during transport of fish by RFID in a fully automated manner. The system allowed: traceability verifying if the range of expected temperature was maintained; information in real time at different links in the distribution chain, and security and quality control along the complex supply chain.

The researches combining TTI-RFID in the cold chain and examples of wireless sensor reinforce the use of RFID in processing steps in food industries. Furthermore, RFID system comprises a reader that uses electromagnetic waves to communicate with an RFID tag by antenna (Fig. I-2), features very similar to wireless sensor. Combining both technologies can provide the following advantages (Mejjaouli and Babiceanu, 2015): increased transportation control; reduced costs related to late delivery; increased agility and responsiveness in face of disturbances related to the spoilage of materials and products during transportation; significantly reduced delivery of unacceptable quality materials and products to customers. However, the advantages were raised under traceability context; they can show the great potential of use RFID inside industries.

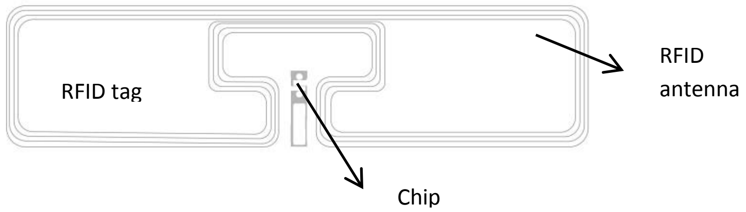

Fig. I-2. Scheme of RFID tag.

The application of RFID in food industries is well exemplified by the use of sensor electrical properties to monitor environment. This is the research line of our group aiming at the use of organic and renewable materials, such as proteins to produce cheaper sensor-enable RFID tags (Bibi et al., 2016a). Our earlier results with RFID coupled with biomaterial have shown the feasibility of this method to monitor the temperature from 20°C to 80°C.

4.1. Challenges of RFID application

Even though it brings several benefits, the fully use of RFID demands to overcome several challenges, such as:

- Sensors need protection against damage; it can affect the way they respond to changes in the environment (Badia-Melis et al., 2014). It forces the necessity of cheap sensors development without mandatory necessity of reuse.
- Inside wireless and RFID nodes context, the response of temperature sensors receives influence depending on the way they are housed (Badia-Melis et al., 2014).

- Interferences take place when there are multiple tags to a single reader's query affecting the responsiveness of the system and also interference of queries of multiple readers to a single tag. Interferences come also from low signal power of weak tag responses compared to stronger neighbour readers' transmissions. The first interference influences the time of response and the others interfere the positioning accuracy (Papapostolou and Chaouchi, 2011). There are many reserches to solve these questions and the results point to a not taylor-made solution but to principles that should be adapted to any different condition of use.
- Metal or glass, that are non-conductive materials, have shielding and reflection effects which affect transduction, especially at Ultra High Frequency Band (UHF) in which more power is used, reducing the capacity to pass through materials (Brody et al., 2008, Laniel et al., 2011, Ruiz-Garcia and Lunadei, 2011).
- The system creates a great amount of data that are difficult to manage (Ruiz-Garcia and Lunadei, 2011).

However, the main challenges are the water interference and the cost that can limit the maximum number of sensors that must be used (Amador and Emond, 2010). Despite of many advantages of RFID technology, the additional costs has been preventing its adoption by the companies (Badia-Melis et al., 2015). Passive tags are less expensive once their power supply comes from the radio frequency field; they just backscatter the carrier signal received from a reader. This makes their lifetime large and cost negligible, contrary to active one that uses a battery (Papapostolou and Chaouchi, 2011). However, depending on the types of RFID tags, it could be of low cost (Bibi et al., 2016a). The tendency is to decrease the price following the application increase and development of this technology.

The RFID system can be operated in several frequency bands, but the most used is Ultra High Frequency because of many advantages, such as: transfer data faster than low and high-frequencies (Ruiz-Garcia and Lunadei, 2011),longer communication distance, higher data rates, as well as smaller antenna size in RFID systems (Sun et al., 2010). But the frequencies normally used in UHF RFID system (915 MHz, 868 MHz) suffer reduction of the readability in ambient close to perishable products with high-water content; water absorbs radio frequency energy decreasing the reading range interfering in the location of the sensors (Amador and

Emond, 2010).However, the presence of water may enable to measure the stimulus influenced by water polarization (Bibi et al., 2016a).

RFID technology uses electromagnetic waves for communication, reason for possible metal and water interference (Papapostolou and Chaouchi, 2011). In food industries, the presence of metals and liquids is inherent to almost food processing. But as the processes are well standardized, the environmental influence of water will be also standardized. The challenge will be to develop a sensor with good sensitivity in order to produce properly monitoring of temperature. Once food has high humidity, studies should be performed to enable this technology (Brody et al., 2008, Laniel et al., 2011).

To overcome the metal interference such as in meat cooking that uses a metallic oven, the location of the antenna inside linked to the reader by a radio frequency cable could overcome the limitation of metal shield. This procedure makes sense based on the available RFID tags with a probe whose reading may be made from outside of the compartment (Amador and Emond 2010).

Despite all challenges and limitations, the use of RFID in food industry provides new tool for temperature monitoring that tends to be low-cost and improves the efficiency of operations and data accuracy (Ruiz-Garcia and Lunadei, 2011). A higher use of RIFD in food industries brings the necessity of integrating food science knowledge for the development of intelligent packaging applications seeking the quality and food safety (Brody et al., 2008).

As for any technology, there are interferences compromising the results. RFID is a potential tool that can be applied for monitoring temperature, but it still needs more studies in order to establish protocols of use focusing certain aspects, such as minimal number and location of RFID sensors (Amador and Emond, 2010, Jedermann et al., 2009). It is mandatory to know the key noise parameters (KNPs) and their ranges and to develop approaches for their elimination. As processes in food industries are standardized, it is easy to recognize and to overcome the KNPs.

Finally, the adoption of wireless technology has not been as fast as it could. Besides, the technical aspects, some reasons mentioned in the past are practically the same 10 years later (Wang et al., 2006, Reyes et al., 2016): a) standardization is not yet complete; b) potential users still wait for reliable results; c) lack of local structure and processes to utilize its full potential; d) complexity and high cost are barriers for introduction in large facilities; e) the reliability of wireless system still needs to be solidified and is considered risk for process control; and f) lack of experienced personnel for troubleshooting.

5. Conclusions

The simplicity of use and of being a well-established technology makes thermocouples widely applied to monitor temperature in industries. However, their disadvantages were the drive force to propose potential new methods focusing mainly on non-invasive, non-contact and on line approaches. The use of wireless sensor has been already tested with success. After packaging (post-processing), the products may be efficiently monitored by TTIs that may be coupled with RFID tags (TTI-RFID) conferring the wireless feature. The feasibility of TTI-RFID and of wireless sensor leads to the use of RFID system to monitor both processing and post-processing. This technology is used mainly for traceability in the cold chain. However, sensor-enabled RFID tags for monitoring temperature based on wireless sensor and TTI-RFID results could be the next generation for temperature monitoring, integrating food industries from production process to the market. Nowadays, with the increase concerning about food safety, both industrial and retail players are stakeholders sharing obligations and responsibilities. This integration opens possibility to monitor temperature in the production chain but also to monitor the same product in each step. It will permit to know the fails and from where they come, facilitating the management; it avoids losses and food spoilage. Moreover, as temperature is a Critical Control Point (CCP), an application of RFID system opens a possibility for effectiveness and integration of a food safety program.

Part II: Selecting of sensing biomaterial

POTENTIAL USE OF GELATIN, SODIUM CASEINATE AND SOYBEAN ISOLATED PROTEIN TEMPERATURE SENSOR BASED ON ELECTRICAL PROPERTIES

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Abstract

The electrical properties of gelatin, sodium caseinate and soybean isolated protein vary according to temperature. The electrical capacitance was determined by sample coated onto interdigital capacitors technique (IDC system) under frequency band of 300 to 900 MHz, temperature range of 20°C to 80°C and 20% to 90% RH, focusing possible application in meat cooking. By a 3^3 factorial experimental design (temperature, humidity and biomaterial as factors), at 868 MHz, the effects of temperature have shown significance ($p < 0.05$) only at high humidity (90% RH) because of water polarization. For all biopolymers, the difference was significant between 80°C and 20°C; but, only for gelatin, it was significant between 50°C and 20°C. However, for gelatin between 50°C and 80°C, the capacitance decreased. By the sample self-supported technique, the influence of water content on the real permittivity and on the loss factor was studied under water activity (a_w) varying from 0.38 to 0.87 and frequency band from 1 MHz to 1.8 GHz. At low a_w, the real permittivity was stable up to the UHF zone and varied according to the frequency band at high a_W for all proteins. It was concluded that all proteins are suitable for use as biosensor, with gelatin being the most sensitive at 90% RH and temperature range from 20° to 80°C.

Keywords: Temperature sensor; Ultra High Frequency; Temperature monitoring; Humidity monitoring; Electrical capacitance; Permittivity

1- Introduction

Biopolymers are abundant, renewable and used in a wide range of technical applications. Because of the film forming ability, they are potential substitutes to synthetic materials used in food preservation and food packaging (Bergo and Sobral, 2007, Mudhoo, 2011, Landi et al., 2015).

Regarding to proteins, their humidity and temperature dependences, mainly studied on gas and vapor transfer properties, permit the use in the field of selective materials, active materials, and self-adjusted material.

The complexity and inhomogeneous structure of proteins together with different origins make difficult to determine their electrical properties (Pitera et al., 2001, Berkowitz and J. Houde, 2015, Marzec and Warchoł, 2005). It is reported that the ability to store energy (real permittivity (ε')) and to dissipate electrical energy (imaginary component (ε'')) define the biopolymers as non-ideal capacitors (Ahmed et al., 2008), turning mandatory to take the electrical properties at frequencies and ambient of interest. Besides, the capacitance dependence on the external stimulus makes the sensors easier to implement, and their use has become extended (Venkatesh and Raghavan, 2004, Büyüköztürk et al., 2006, Rittersma, 2002).

Soybean isolated protein (SIP) is the high-quality and low cost alternative to animal protein (Shen et al., 2015). Gelatin and sodium caseinate have been widely investigated for packaging applications (Kadam et al., 2015). Research on electrical properties of these proteins is reported: dielectric constant of SPI was concentration, temperature and frequency dependent (Ahmed et al., 2008). Conductivity of caseinate may be applied in sensors for milk quality control (Mabrook and Petty, 2003). Gelatin, alone or combined with other materials, is used for: biomedical applications (Kanungo et al., 2013); denaturation process (Kubisz and Mielcarek, 2005); microwave sensor for water activity (Clerjon et al., 2003); biodegradable low-cost energy storage (Landi et al., 2015).

Our research group has been studying the electrical properties of biopolymers to investigate how these properties depend on the temperature and humidity (Bibi et al., 2016a). Electrical properties of proteins have been largely studied when dispersed into solution, but the literature is relatively scarce concerning protein based material, which is worse when considering variation with temperature and/or humidity on a large frequency range. This dependence might be of interest in the field of intelligent packaging biosensor to indicate temperature and/or humidity changes featuring an innovative and unusual application of biosensor.

The expected use of biosensors is to detect analytes, but the electrical properties of proteins will be evaluated to elaborate sensing biomaterial for temperature indicator. Proteins are good candidates because of their sustainability coming from renewable resources and also as by-products of food industry. Another advantage is the potential use for both temperature monitoring and food quality makers.

Aiming at the use of gelatin, of sodium caseinate and of soybean isolated protein as biosensor of temperature, the electrical properties were studied as a function of different temperatures and humidity referenced by meat cooking parameters and frequency range of 1 MHz up to 1.8 GHz. This work is part of an ongoing project focusing on the use of biopolymers as a temperature biosensor in RFID systems.

2. Material and Methods

The electrical properties were studied under temperature and humidity conditions normally used in meat cooking (cook-in process). Two techniques were used: electrical capacitance of sample coated onto interdigital electrodes (IDC system) (Bibi et al., 2016a) and real permittivity and loss factor of self-supported sample (volumetric capacitance) to evaluate the biopolymers.

2.1. Thermogravimetric Analysis (TGA)

Thermogravimetric analyses (TG/DTG) of the powder of soybean protein isolated, sodium caseinate and gelatin were ran in a TGA, model Pyris 1, Perkin-Elmer. The carrier gas was nitrogen at a flow rate of 30 mL/min. The temperature range was 20°C to 80°C, at a heating rate of 10°C/min. The analyses were performed in duplicate.

2.2. Preparing the solution

Soybean protein isolated, sodium caseinate and gelatin were provided by Seah International (Wimille France), Bel Industry (Vendôme, France) and Merk (Darmstadt, Germany), respectively. The concentration used was 10% w/v (H_2O), prepared as shown by (Arfa et al., 2007, Helal et al., 2012, Fakhoury et al., 2012). The bubbles dissolved in the solutions were removed under vacuum.

2.3. Thickness

The average thickness of the samples was measured at the center and at four opposite positions by a hand-held digital micrometer (0.001 mm) model MDH-25M (Sakado, Japan). All samples were measured after coating onto the interdigital electrode (sample coated) and dried sample (self-supporting).

2.4. Samples Coated onto Interdigital Electrodes

2.4.1. Preparing Samples

The solutions were coated onto the surface of the interdigital electrodes with circuit reference of 1 GHz (Cirly, France), by the film applicator Coatmaster 510 (Erichsen, Germany), followed by a drying step at room temperature and relative humidity (around 25°C and 50%, respectively). A blank uncoated electrode was also used as a reference.

2.4.2. Statistical Analyses

A 3^3 factorial design with three central points was used. The levels were: 20%, 55% and 90% for humidity, and 20°C, 50°C and 80°C for temperature for each protein studied: gelatin, sodium caseinate and soybean isolated protein. The variable of answer was the electrical capacitance.

For all statistical analyses, it was used 5% as a significant level and the Statistica software, for Windows, version 12.0 (Tulsa, USA). All data are presented as average values ± standard deviations ± 1.

2.4.3. Determination of the electrical capacitance

The electrical capacitance was determined according to Gervogian model (Wang et al., 2003). It was used Impedance Analyser HP 4191A RF (Agilant, USA), at a frequency range of 300 to 900 MHz, that was linked to interdigital electrodes by a coaxial cable semi-rigid SMA (Amphenol Connex, France) and to the connector coaxial SMA 500HM Solder SMA (Amphenol Connex, France). The temperature and humidity were controlled by a climatic chamber (Secasi Technologies, France). For the measurements, the sample was conditioned at 20% RH and the temperatures varied (20°C, 50°C and 80°C). The process was repeated at 55% and 90% RH. The software used to record the results was LabView (National Instruments, USA).

2.5. Self-supporting Samples

2.5.1. Preparing the Samples

The solutions were poured in aplastic container to form a wet film, with a thickness of approximately 0.8 cm. After, they were dried at room temperature and relative humidity of 60%. They were cut with a borer in order to get samples with 2 cm of diameter (before being completely dried). A teflon film was also used as a reference.

2.5.2. Humidity conditioning

The humidity dependence was evaluated at two different water activities (a_w). The samples were conditioned in desiccators with saturated solutions of potassium carbonate (111 g/100 mL) and potassium nitrate (47 g/100 mL) salts. The a_w was taken daily in triplicate (FA-st/1, GBX) up to stability. For gelatin, sodium caseinate and soybean isolated protein, the stabilized a_w values were 0.82, 0.87, 0.85 for nitrate carbonate and 0.40, 0.38 and 0.43 for potassium carbonate, respectively.

2.5.3. Determining the Volumetric Capacitance

The response variables were dielectric constant and the loss factor, according to the equation:

$$C = \frac{\varepsilon_r \varepsilon_0 A}{d}$$

where C is the capacitance, ε_r is the relative permittivity, ε_0 is the vacuum permittivity (8.85×10^{-12} F/m), *A* is the electrode surface, and *d* is the distance between the two electrodes (sample thickness).

The Impedance Material Analyser HP 4291B (Agilent, USA) was used from 1 MHz to 1.8 GHz. Five measurements were performed for each sample.

3. Results and Discussion

3.1. Thermogravimetric analysis (TG)

In the first stage, ΔY_1 indicates free water loss, whose values were (9.5 $\pm$ 0.2)%, (12.5 $\pm$ 0.6)% and (8.4 $\pm$ 0.9)% for sodium caseinate, gelatin and soybean isolated protein, respectively. For the second stage, ΔY_2 is related to a material degradation, whose values were (68.5 $\pm$ 4.9)%, (74.5 $\pm$ 8.7)% and (67.4 $\pm$ 5.7)% for sodium caseinate, gelatin and soybean isolated protein, respectively (Fig. II-1).

The onset degradation temperatures, taken just after the first stage, were (290.4 $\pm$ 11.9)°C, (315.7 $\pm$ 3.6)°C and (286.1 $\pm$ 0.6)°C for sodium caseinate, gelatin and soybean isolated protein, respectively, which are in agreement with other studies from the literature: between 280-285°C (Yu et al., 2010), 289.74°C (Ahmad et al., 2015) and 270-280°C (Siva Mohan Reddy et al., 2014), respectively. For all proteins, there is no evidence of degradation/oxidation at temperature normally used for meat cooking (80°C). Higher values mean higher stabilities (Jain and Sharma, 2011); thus, gelatin was the most stable biomaterial.

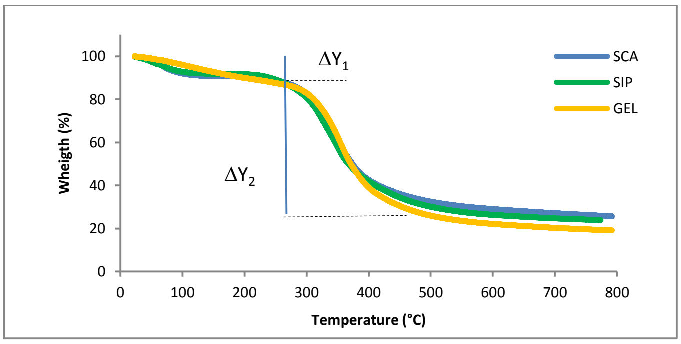

Fig. II-1. Thermogravimetric analysis of sodium caseinate (SCA), gelatin (GEL) and soybean isolated protein (SIP).

3.2. Sample coated onto the interdigital electrode (IDC)

The IDC system was used to evaluate the electrical capacitance. It is characterized by their simple design, low installation height, and inexpensive manufacturing (Jungreuthmayer et al., 2012). There are several sensor applications, such as food packaged quality (Tan et al., 2007); bacterial growth (Ong et al., 2002), meat inspection (Mukhopadhyay and Gooneratne, 2007), and RFID (Jungreuthmayer et al., 2012).

Temperature and humidity were statistically significant ($p<0.05$) for the whole Ultra High Frequency (UHF) band studied (300-900 MHz). However, the type of proteins was only statistically significant after 604 MHz. The data show that the quadratic effect of humidity is significant, pointing to a higher influence of this parameter on the electrical properties.

3.2.1. Effect of frequency on the electrical properties of proteins

The electrical properties of materials are dependent on their chemical composition and on the permanent dipole moments associated with water (Venkatesh and Raghavan, 2004). The blank uncoated electrode practically did not change with frequency, independent of humidity and temperature, what indicates that the sensing response is related to the biomaterial films (Fig. II-2).

The electrical capacitance of all biopolymers was frequency dependent; it increases as frequency increases. This behavior was more intense at higher humidity (90% RH), as shown in Fig. II-2, what agrees with literature (Ryynänen, 1995, Zhu et al., 2010).

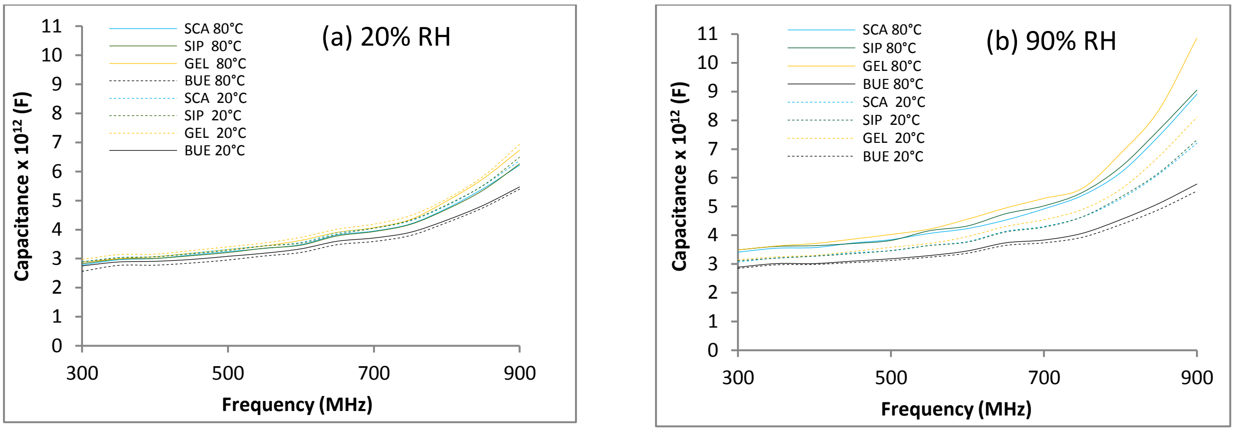

Fig. II-2. Effect of frequency on the capacitance of sodium caseinate (SCA) film of 54 µm, soybean isolated protein (SIP) film of 56 µm, gelatin (GEL) film of 57 µm and blank uncoated electrode (BUE), at (a) 20% RH and (b) 90% RH. Experiments made in triplicate with coefficient of variation below 10%.

Gelatin has shown the highest value of capacitance; while for soybean isolated protein and sodium caseinate, the electrical answers were very similar. The higher molecular weight of gelatin (300 kDa (Figueiró et al., 2004) compared to 20.2 to 81.4 kDa for soybean isolated protein (Martins, 2005) and 23 kDa for sodium caseinate (Gubbins et al., 2003), can explain its better performance, once this parameter can change the electrical behavior (Kolesov, 1968).

However, the molecular size of gelatin (length of 300 nm and diameter of 1.5 nm (Figueiró et al., 2004) is much smaller than the wavelength at UHF (1 m-10 cm) (Sanghera and Thornton, 2007), leading to a dependence of electrical properties only on the shape (Ryynänen, 1995).

Difference in molecular shapes leads to different surface areas, interfacial areas and interfacial polarization; consequently, the dielectric properties change (Dang et al., 2012). The main characteristic of gelatin is the triple-helical domains (Rest et al., 1993), that is stabilized by both interchain hydrogen bonds and 'structural' water molecules (Sarti and Scandola, 1995).The ability of its unfolded peptide chains to trap a large amount of water molecules allows to utilize their intrinsic dielectric properties (Sanwlani et al., 2011). Even though the large number of C=O…H-N hydrogen bonds limits the mobility of the polarized groups, they are on a large number, what is beneficial for the polarization (Ning et al., 2015).

3.2.2. Effect of temperature and humidity on electrical properties of proteins at 868 MHz

The frequency dependence of the biopolymers (Fig. II-2) is strictly linked to polarisation (Venkatesh and Raghavan, 2004, Büyüköztürk et al., 2006). Only from 604 MHz, the influence of biopolymers on the capacitance was significant, but for the analysis herein, it was considered a frequency of 868 MHz, as it is the one applied in RFID system in Europe (Bibi et al., 2016a).

The sensitivity to environmental conditions, such as temperature and relative humidity, is reported as a very important restriction factor concerning the films because they are hydrophilic materials and thus very susceptible to water (Gennadios, 1993). The sensitivity to water vapor is reported as the largest challenge for their practical applications (Potyrailo et al., 2011). However, in our work, this sensitivity was suitable as it has changed the electrical properties indicating the humidity and temperature dependence.

Both temperature and humidity influence the capacitance (Foucaran et al., 2000). But up to 55% RH, the curves were very similar and there was a quite stable proportionality regarding to the blank uncoated electrode, aspects that change sharply at 90% RH (Fig. II-3), because of the influence of water polarization. This is a desired condition, once the humidity used during the meat cooking is around 90-95%. Regarding to 90% RH, the Fisher's test shows that, for SIP, GEL and SCA, there was a statistical significant difference ($p < 0.05$) only between 20^{o}C and 80^{o}C. However, only gelatin was able to have a statistical significant difference for capacitance between 20^{o}C and 50^{o}C, a temperature range common in food industries.

It is reported that an applied electrical field inducts polarization currents in the biological macromolecule (Greenebaum, 2006). At UHF, it occurs the following types: a) ionic, that takes place where the hydrated ions try to move in the direction of the electrical field, transferring energy by this movement; b) electronic, that is characteristic of all substances and orientation or dipolar, because of the dipoles absorption of H_2O, besides being strongly temperature dependent, that was clearly observed by the temperature versus humidity curves (Fig. II-3) (Blakemore, 1985, Chani et al., 2013, Ryynänen, 1995).

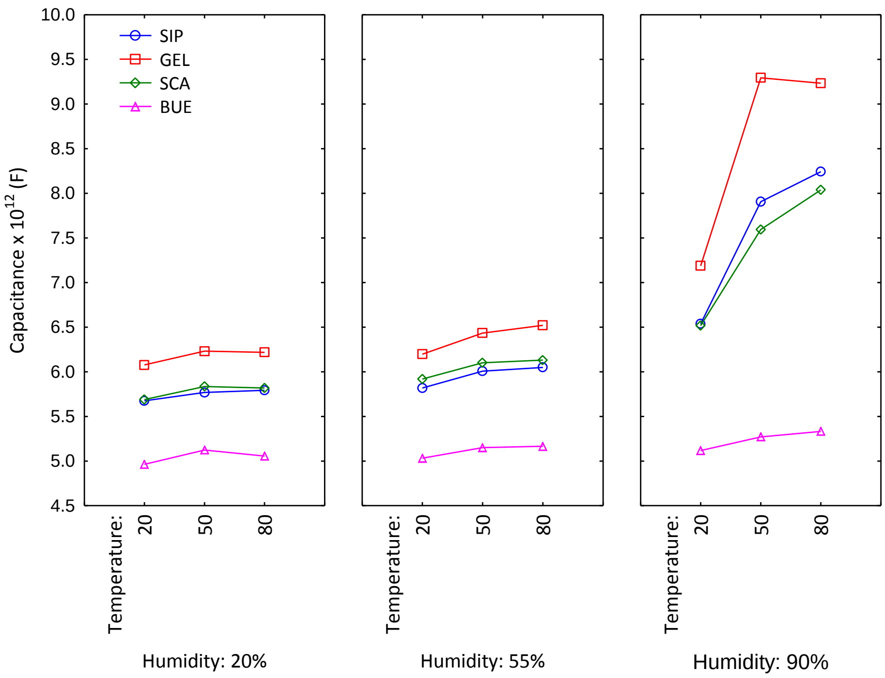

Fig. II-3. Influence of temperature (20°C, 50°C and 90°C) and humidity (20%, 55% and 90%) on the capacitance for soybean isolated protein (SIP), gelatin (GEL) and sodium caseinate (SCA) and blank uncoated (BUE), at a frequency of 868 MHz. All coefficients of variation were lower than 10%.

The orientational polarization increases with increasing temperature. At low values, the dipoles in polar materials cannot orient themselves (Ashry et al., 2009). The capacitance increases as temperature increases at 20% RH and 55% RH; but only at 90% RH the effect was remarkable showing the effectiveness of monitoring temperature only at high humidity.

Considering 90% RH, for 20ºC-50ºC, there is a deeper increase of the capacitance with temperature compared to 50ºC-80ºC. Maybe, this can be explained by the glass transition temperature (T_g) of these biopolymers. For gelatin, indeed, the capacitance drops after 50ºC, indicating that this material is more sensitive to T_g. When above the glass transition temperature, the molecules will be rearranged and there will be some free volume among them, influencing the electrical properties (Schneuwly et al., 1998).

In order to overcome the reduction in the capacitance derivative with temperature at high humidity and temperature range of 50°C-80°C, other variables should be considered, such as reducing frequency (Kanungo et al., 2013) and reducing layer thickness (Story et al., 1995).

The water mobility is a basic condition to change the electric properties (Ahmed et al., 2008) and the water permeability does not change on heating protein films without plasticisers (Micard et al., 2000), condition used herein. Once the capacitance increased as humidity increased, the behavior of the biopolymers agreed with the current knowledge. However, humidity can increase the mass and layer thickness, variables that can also influence the electrical properties (Zhu et al., 2010, Story et al., 1995).

The results have shown feasibility for using biosensors as a temperature indicator at UHF, that permits longer communication distance, higher data capacity and smaller antenna size in RFID systems (Sun et al., 2010).

3.3. Self-supported samples (volumetric capacitance)

The self-supported sample characterizes the biomaterial. This technique was applied by changing the frequency and humidity (the most important variable that influenced the electrical properties). The results are presented in Fig. II-4 and Table II-1.

All biopolymers were humidity dependent. The curves under different water activity (a_W) presented different shapes. However, they were similar for sodium caseinate and soybean isolated protein; gelatin showed higher sensitivity to high humidity (Fig. II-4). Taking as reference 600 MHz, it is possible to see that the real permittivity of gelatin is very large compared to the other biopolymers. This behavior can be confirmed by the variation (Δ) of real permittivity, of loss factor and of frequency at resonant frequency (RF) (Table II-1). For teflon as a reference, these variables were, respectively, 114, 222 and 1,620 MHz, showing that the electrical answers were caused by biomaterial sensitivity.

The frequency at RF was very similar for all biopolymers, except for gelatin at higher a_W. The value was smaller comparing to soybean isolated protein and sodium caseinate (Table II-1). It means that even though presenting higher sensitivity considering the frequency band studied (1-1.8 GHz), the gelatin was less stable. It agrees with aforementioned higher tendency of a reduction in capacitance under high humidity (90% RH) (Fig. II-2).

Table II-1. Values of the frequency, real permittivity and loss factor for gelatin (GEL), sodium caseinate (SCA) and soybean isolated protein (SIP) at the resonant frequency (RF).

	GEL			SCA			SIP		
	Water activity			Water activity			Water activity		
	0.82 ± 0.02	**0.40 ± 0.04**	**Δ**	**0.87 ± 0.04**	**0.38 ± 0.05**	**Δ**	**0.85 ± 0.02**	**0.43 ± 0.05**	**Δ**
Frequency (MHz)	600± 0.09	1,112± 0.81	**512**	760± 0.15	1,190± 0.01	**430**	790± 1.2	1,250± 0.12	**460**
Real permittivity	31 ± 1.4	44 ± 1.5	**-13**	27 ± 3.1	57 ± 2.3	**-30**	23 ± 3.89	57 ± 1.2	**-34**
Loss factor	49 ± 2.3	75 ± 3.9	**-26**	42 ± 1.9	97 ± 1.0	**-55**	37 ± 1.9	98 ± 4.3	**-61**

The RF increased at lower a_W because of less water adsorbed, as was pointed out by researches with graphene (Zhu et al., 2010). Comparing also to the curves at higher a_W, there is a shift of RF to higher values. As there is more available water, there is also more mass to be moved, situation that implies in lower RF by atomic polarization that is closely related to electronic polarization (Ryynänen, 1995). This shift may imply in higher stability with lower water content, although there is less sensitivity that can limit the use of the biosensor, as shown in Fig. II-2.

For the samples at lower a_W, the real permittivity was practically the same up to the beginning of the UHF band (300 MHz - 3 GHz, in Fig. II-4). The values were always lower than 10. The reason is due to presence of electronic and atomic polarizations. When only these two mechanisms are present, the material is almost lossless at microwave frequencies (Ryynänen, 1995). For all biopolymers, the loss factor was negligible, but before the RF and the microwave frequencies (2.45 to 5.8 GHz in Fig. II-4).

On the contrary, at higher a_W, the real permittivity was frequency dependent. It decreased up to the High Frequency zone (3 to 30 MHz) because of the orientation, electronic and interfacial polarizations at low frequencies (El-Nahass et al., 2014). After, in the Very High Frequency band (30 to 300 MHz), there was a short stabilization, probably because of the interfacial polarization. As the frequency increased, the dipole was completely unable to follow the field and then, the orientation polarization ceased (El-Nahass et al., 2014). At Ultra High Frequency, the permittivity started to increase (Fig. II-4a). These behaviors were the same for all biopolymers, but researches, with dispersion of soybean isolated protein, soybean flour and gelatin, have shown that the permittivity decreases at all UHF bands (Ahmed et al., 2008, Solanki et al., 2016, Guo et al., 2010), in opposition to the solid sample used herein.

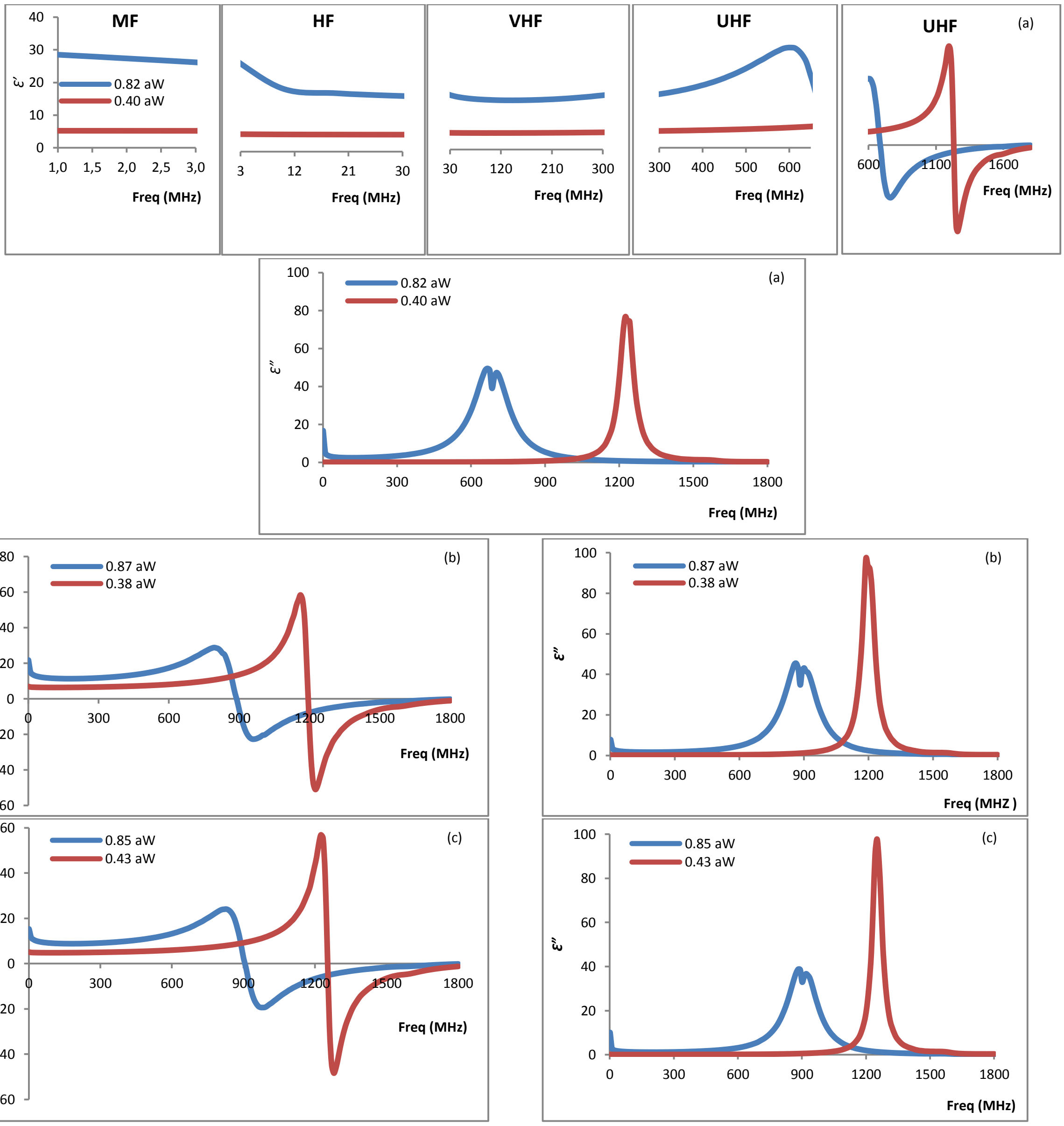

Fig. II-4. Scatterplots showing the real permittivity (Ɛ′) and loss factor (Ɛ″) as a function of frequency (Freq) (varying from 1 MHz to 1.8 GHz) for samples of (a) gelatin, with thickness of 0.46 mm at 25°C and water activity of 0.82 and 0.40; (b) sodium caseinate with thickness of 0.47 mm at 25°C and water activity of 0.87 and 0.38; and (c) soybean isolated protein with thickness of 0.46 mm at 25°C and water activity of 0.85 and 0.43.

4. **Conclusions**

Two different techniques, sample self-supported and coated onto interdigital electrode, were used and both have shown the influence of water polarization. They were able to measure the electrical properties of gelatin, sodium caseinate and soybean isolated protein. The second technique has shown the same tendencies obtained by the first one, showing that the results were robust. The electrical capacitance, real permittivity and loss factor were frequency, temperature and humidity (the most important factor) dependent. Indeed, the influence of temperature was only important at high humidities (from 90% RH). The samples at lower humidity conditions have not presented frequency dependency, mainly before the UHT zone, condition that was opposite with samples at high humidity, that show the importance of water polarization. Based on the results, the use of biosensors to monitor temperature is feasible on account of electrical capacitance sensitivity at 90% RH, which is the same RH used in meat cooking. Gelatin, because of its molecular shape and chemical characteristics, was the most sensitive biosensor. However, further adjustments should be carried out to enlarge its use between 50°C and 80°C, avoiding the tendency of a reduction in the capacitance derivative with temperature at high humidities. This study offers a potential tool for monitoring temperature coupling biological films and RFID system.

Part III: Biomaterial evaluation

FEASIBILITY OF A GELATIN TEMPERATURE SENSOR BASED ON ELECTRICAL CAPACITANCE

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Abstract

The innovative use of gelatin as temperature sensor based on capacitance was studied at temperature range normally used for meat cooking (20°C-80°C). Interdigital electrodes coated by gelatin solution and two sensor thicknesses, 38 and 125 μm, were studied between 300-900 MHz. At 38 μm, the capacitance was adequately measured but for 125 μm the slope capacitance versus temperature curve decreased before 900 MHz, because of the electro-thermal breakdown between 60°C and 80°C. Thus, for 125 μm, the capacitance was studied applying 600 MHz. Sensitivity at 38 μm at 868 MHz (0.045 pF/°C) was lower than 125 μm at 600 MHz (0.14 pF/°C) influencing the results in the simulation (temperature range versus time) of meat cooking; at 125 μm, the sensitivity was greater mainly during chilling steps. The potential of gelatin as temperature sensor was demonstrated and a balance between thickness and frequency should be considerate to increase the sensitivity.

Keywords: sensor, gelatin, temperature control, electrical capacitance, meat cooking

1- Introduction

Temperature measurements are very important for several types of industries. In the food industry, its monitoring is essential to guarantee the food safety; thus, it is a Critical Control Point (CCP). The principal temperature sensors used are thermal resistor, thermal diode and thermocouple (Wang and Tang, 2012), that is the most used because of its reliability and low cost (Zell et al., 2009). Furthermore, the associated limitations related to meat cooking are: monitoring only a few numbers of products in the oven and impossibility to monitor the same product from heating to cooling steps, if they are made separately, and during storage. These features open a window for tools that are able to control both production and distribution, possibilities that can be reached with wireless systems (Abad et al., 2009).

Combining temperature sensor or indicator with an RFID tag can be the best choice for products in the chilling chain (Wan and Knoll, 2016). This application was reported by literature (Abad et al., 2009, Kim et al., 2016b), but its use in other unit operations is scarce. However, for all of them, it is imperative an adequate operation of the sensitive part.

Our research group has been studying biopolymers as environmental sensors, focusing to couple them with RFID tags (Bibi et al., 2016a). In our previous tests, gelatin was suitable as a sensor of temperature at high humidity (90% RH), the same most common heat treatment applied for meat cooking by meat industries (James and James, 2014). Modern biosensors are a combination of biology and electronics and it is a promise for on-line measurements of important process parameters and microbial detection (Ramaswamy et al., 2007).

The temperature indicators normally are based on physical sensors; the use of biomaterial is an innovative proposal, based on the simplicity, low cost, and availability of renewable sources. Coupling it with capacitive technique, that is also of low cost and robust (dos Reis and da Silva Cunha, 2014), may permit a cheap and efficient temperature sensor. The effectiveness of this technique is reported in several applications besides temperature (Mohamed, 2008): volumetric concentration (dos Reis and da Silva Cunha, 2014), moisture (Böhme et al., 2013), DNA detection (Guiducci et al., 2004), and microbiological growth (Li et al., 2011).

Gelatin has potential as a sensor because of its biocompatibility, biodegradability, low cost, and easy manipulation (Gaspard et al., 2007, Klotz et al., 2016). Besides, it is recognized as a safe (GRAS) material, a necessary feature for food industry, and by its high mechanical strength and characteristic of stabilizing agent (Li et al., 2014, Babu et al., 2007). The chemical composition has a large number of polar functional groups that are beneficial to the polarization under electric field (Ning et al., 2015). As a hydrogel, it is able to imbibe large amounts of water and does not dissolve because of chemical or physical crosslinks and/or chain entanglements. Hydrogel responds to environmental changes, such as pH, temperature, and ionic strength (Peppas et al., 2012).

There have been several reports combining gelatin and electrical properties (Kanungo et al., 2013, Kubisz and Mielcarek, 2005, Clerjon et al., 2003, Landi et al., 2015, Mao et al., 2014, Ning et al., 2015) and its use as a biosensor, such as biomedical applications and denaturation process (Zheng et al., 2015, Emregul et al., 2013, Saum et al., 2000, Ebrahimi et al., 2014, Topkaya, 2015, Huang et al., 2010). However, the literature is scarce to report gelatin as a temperature sensor. It was used as a protective and reducing agent for a visual physiological temperature sensor at room temperature (Lan et al., 2015).

The other advantage of gelatin is its potential as sensing material because of different interactions such as H-bonding, hydrophobic interactions, covalent, etc., leading to a biocompatibility with several quality markers (NH_2, COOH, $CONH_2$, OH and SH) (Miyahara et al., 1978, Pulieri et al., 2008, Zeugolis and Raghunath, 2010), showing the great potential to control temperature and food spoilage after production and also in the market.

Engineering the bioelectrochemical sensing interface is crucial for improving its sensitivity (Jia et al., 2016). In the literature, several methods have been used for this purpose, such as concentration of solution (Ahmed et al., 2008), polarization (Chani et al., 2013), addition of PVA (Selestin Raja and Nishad Fathima, 2015), and addition of nanomaterials (Jia et al., 2016). Thickness also influences the sensitivity (Zhu et al., 2010); indeed, this is the simplest variable to manipulate the sensitivity instead of adding components such as before mentioned, what rises the complexity in preparing the sample once homogeneity also influences the electrical properties (Büyüköztürk et al., 2006). Furthermore, working with thickness, keep the simplicity and low cost that are qualities desirable for sensors.

The use of biopolymers to monitor temperature during processing in food industries, such as meat cooking, is a new concept, whose potential was already shown in our previous research (paper to be published soon). In this work, the use of gelatin was investigated in order to determine its feasibility as a temperature sensor and the objectives were: (1) to study the influence of the layer thickness on the electrical capacitance sensitivity, (2) to evaluate its application under meat cooking protocol, and (3) to evaluate its stability for continuous use of the same sensor.

2. Material and Methods

The electrical properties of gelatin were explored considering a temperature range of 20°C to 80°C and humidity of 90% RH, conditions normally used in meat cooking processing, as it allows to obtain water activity values close to the meat products (0.93 to 0.97). The frequency band studied was 300 MHz up to 900 MHz, focusing analysis at 868 MHz, that is the frequency used for the European UHF RFID (Bibi et al., 2016a).

2.1. Differential scanning calorimetry (DSC)

The thermal analysis of the gelatin was carried out in a DSC from Perkin-Elmer, model Diamond, with an external refrigerating device (Intercooler II) and nitrogen as a purge gas system, with a flow rate of 20 $mL \cdot min^{-1}$. The temperature range was 25°C–170°C, at a heating rate of 10°C/min. The analyses were made in triplicate.

2.2. Scanning Electron Microscopy (SEM)

The SEM analysis was carried out in a FEI Quanta 200 FEG. It is equipped with **X-Max**^{50mm2} (Silicon Drift Detector), manufactured by Oxford Instruments. The sample was composed by gelatin coated IDC system on the SEM stubs.

2.3. Thickness

The average sample thickness was measured at the center and at four opposite positions by a hand-held digital micrometer (0.001 mm). All samples were measured after coating onto the interdigital electrode. The experiments were made with 38 μm μm ± 1 (value close to the thickness of electrode – around 30 μm) and 125 μm μm ± 2 (reference value to the technical limit to cast the sample). Samples with thickness of 61 μm μm ± 1 and 84 μm μm ± 2 were used only for comparison.

2.4. Solution preparation

It was used gelatin (Merk) with the following physical-chemical composition: pH (3.8-7.6); SO_2 (< 0.005%); arsenic (< 0.0001%); heavy metals (< 0.001%); peroxide (< 0.01%); phenolic preservatives (undetectable); sulphate dash (< 20%); grain size - 800 μm (99%). The concentration used was 10% w/v and the solution was prepared as shown by (Fakhoury et al., 2012). The bubbles dissolved in the solutions were removed by vacuum conditions.

2.5. Determination of electrical properties

Electrical properties were studied by sample coated onto the interdigitate electrode (IDC) technique and the response variable was electrical capacitance determined according to Gervogian model (Wang et al., 2003).

2.5.1. Preparing samples

The solution coated the surface of the interdigitate electrodes, with a circuit reference of 1 GHz (Cirly, France), by using the film applicator Coatmaster 510 (Erichsen, Germany), followed by a drying process at room temperature. A blank uncoated electrode was also used as a reference. IDC was used because of their versatile use in different environmental conditions and on a large scale of frequency (Bibi et al., 2016a).

2.5.2. Determination of the electrical capacitance

The determination of the electrical capacitance was made in triplicate. It was used the Impedance Analyser HP 4191A RF, at a frequency range of 300 to 900 MHz, and 500mV for the oscillator voltage, that was linked to interdigital electrodes by a coaxial cable semi-rigid SMA (Amphenol Connex, France) and the connector coaxial SMA 500HM Solder SMA

(Amphenol Connex, France) (Fig. III-1). The temperature and humidity were controlled by a climatic chamber (Espec, Japan). The measurements were made at temperatures of 40°C up to 80°C and at 90% RH, after stabilization of electrical capacitance. The time was calculated between the moment before changing the temperature and the moment just after started the next stabilization of capacitance. An application test was made according to protocol of meat cooking (Fig. III-5). The software used to record the results was LabView (National Instruments).

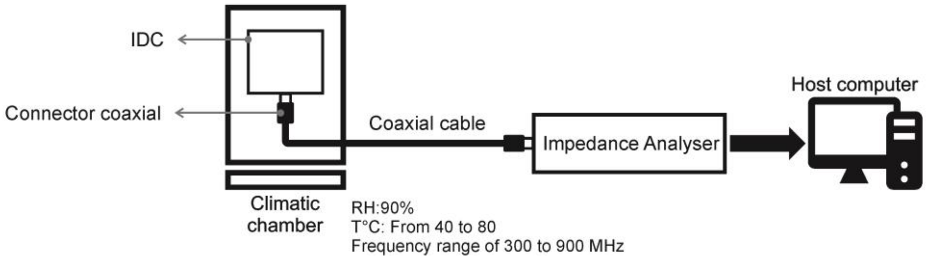


Fig. III-1. Experimental set-up used for the electrical capacitance tests. IDC: interdigitate electrode.

3. Results and discussion

3.1. Effect of temperature and thickness on the electrical capacitance

In our previous researches (paper to be published soon), with samples up to thickness around 50 μm, the electrical capacitance was adequately measured at experimental conditions: 90% RH, 20°C up to 80°C and 300 MHz up to 900 MHz. This stability was also observed for sample with 38 μm up to 900 MHz, but for the samples with higher thickness (61 μm and 125 μm), the curve of capacitance dropped before 900 MHz, in a value that was lower for higher temperature (Fig. III-2).

Each dielectric shows a characteristic behavior as a function of frequency and temperature (Schneuwly et al., 1998). The material can be ionized and become a conductor as no dielectric material is a perfect insulator. Trace amount of electrical conduction is always present, especially at high electric field and/or elevated temperature (Li et al., 2015). This tendency can be related to the dielectric strength according to the theories of Artbauer and electro-thermal breakdown (Li et al.).

Both theories are based on the influence of temperature, variable that was assumed to explain the final stage of a breakdown process (Ho and Jow, 2012). It can be seen that with a constant frequency (868 MHz), there was a conductor effect between 60°C and 80°C for the samples at 61 μm and 84 μm that has finished after returning to 60°C (Fig.III-3).

In the electro-thermal breakdown, above certain voltage, heat cannot be removed from the dielectric as rapidly as it is generated, which results in thermal breakdown (Xiaoguang Qi; Zhong Zheng; Boggs, 2003). Thus, the critical conductivity will be attained under lower electric field when the temperature is higher. Consequently, the breakdown field decreases with the increase of temperature.

In Artbauer's theory, the temperature dependence on the dielectric strength is understood in terms of the effect of temperature on the free volume and molecular relaxation process. When above the glass transition temperature, the molecules will be rearranged and there will be some free volume among them. The breakdown is influenced by the motion of charge carriers through voids polymer arising from its free volume. The temperature increase leads to an increase of the available free volume and to larger void dimensions. Thus, the breakdown is easier to happen when temperature rises (Schneuwly et al., 1998).

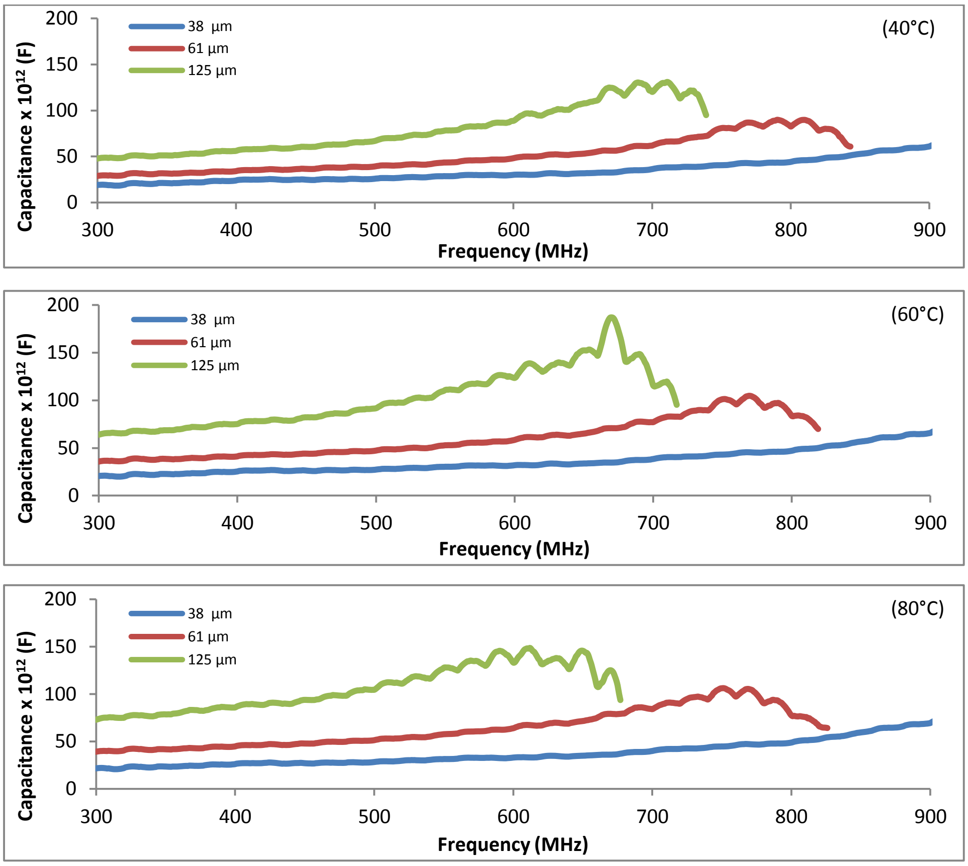


Fig. III-2. Influence of frequency (300-900 MHz) on the electrical capacitance of gelatin, with thickness of 38, 61 and 125 µm, for temperatures equal to 40°C, 60°C and 80°C. Experiments made in triplicate with coefficient of variation below 10%.

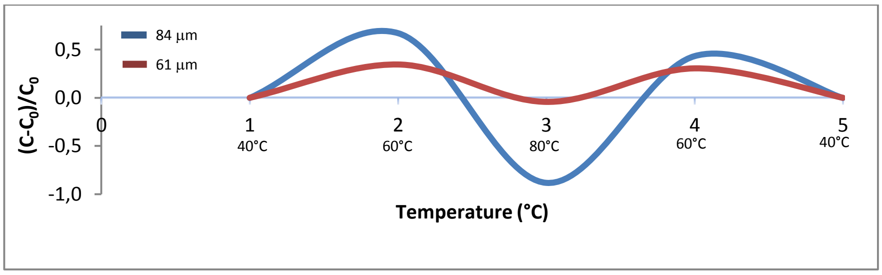


Fig. III-3. Effect of temperature on the capacitance of gelatin (expressed by $(C-C_0)/C_0$): thickness of 84 µm and 61 µm at 868 MHz and humidity of 90% RH. C (capacitance at 40°C, 60°C, 80°C); C_0 (capacitance at 40°C).

This theory was confirmed in DSC measurements of polypropylene foils that have revealed strong correlation between structural phase transitions at the same temperature regions as it shows discontinuities in the breakdown strength (Schneuwly et al., 1998). The same was observed with the electrical properties of gelatin, once between 60°C and 80°C the curve of capacitance dropped (Fig. III-3) and it is quite the same band of temperature where the glass transition temperature T_g started and finished, whose extrapolated value is 77.84 ± 0.13°C (Fig. III-4).

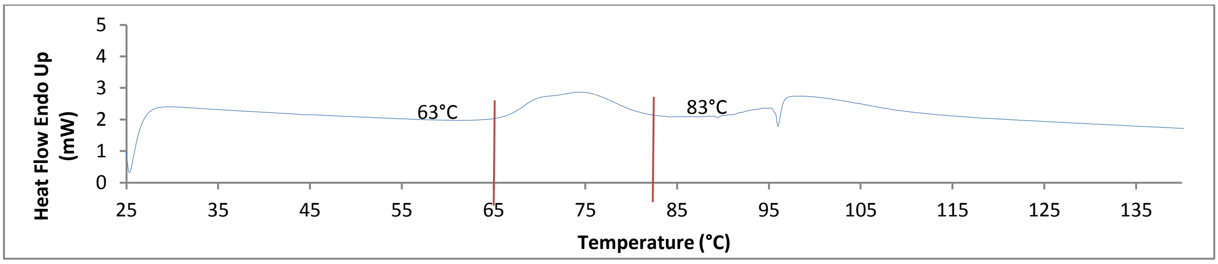


Fig. III-4. Result of differential scanning calorimetry (DSC) analyses of gelatin.

Both theories mention and explain the breakdown temperature dependence that appeared only for thicker samples (61 µm and 125 µm) showing that thickness is also a variable that influences the electro-thermal breakdown (Schneider et al., 2015). To work with thicker samples, it may be used the given frequency value obtained just before the capacitance starts decreasing; in our case, this value was lower than 868 MHz, that it is the frequency used by the European System UHF RFID (Bibi et al., 2016a) (Fig. III-3).

The thickness affects the sensitivity, but there is a limit considering loss of linearity at higher values (Zhu et al., 2010). These are supported by studies with IDC and polyimide as a sensor (Story et al., 1995, Boltshauser et al., 1991). Thus, these results point out to the necessity of a good balance between thickness and frequency to the adequate use of gelatin sensor. Based on our early studies with real permittivity of gelatin, 600 MHz was the highest frequency for better readability before reaching the resonant frequency value and it was chosen to the following studies with sample with 125 µm.

A utilization of the sensor with thick layer at 868 MHz may be considered in applications whose maximum temperature is lower than 60°C, as mentioned earlier. In addition, although humidity was not a variable studied herein, in an essay at 45% RH and at 868 MHz, the slope of a capacitance versus temperature curve did not decrease. It may permit the use of thicker gelatin sensor in environmental with reduced temperature range or under low humidity.

3.3. Hysteresis and sensitivity

The hysteresis of gelatin samples with 38 μm and 125 μm were shown in Fig. III-5. The temperature range of 40°C up to 80°C was studied once in this interval the instability of the capacitance measurements were normally observed.

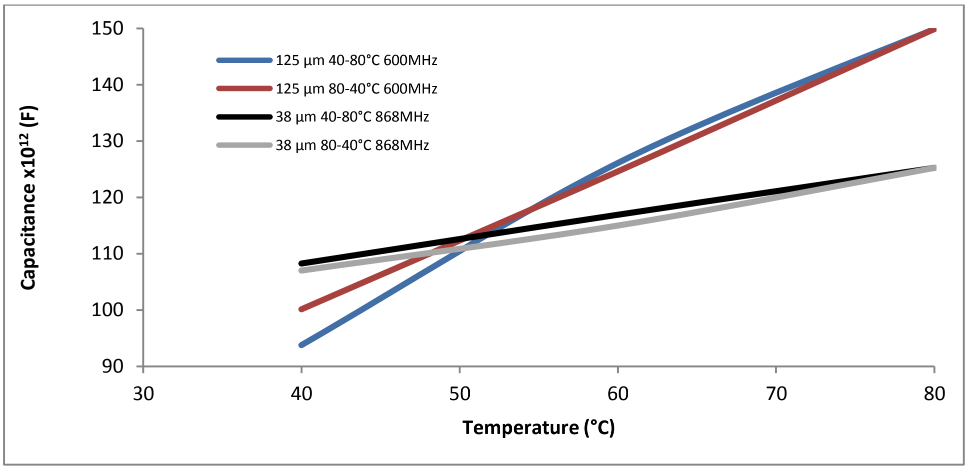


Fig. III-5. Hysteresis of gelatin from 40°C to 80°C and 90%RHfortwo thicknesses: 125 μm (600 MHz) and 38 μm (868 MHz). Experiments made in triplicate with coefficient of variation below 10%.

Both curves (40-80°C and 80-40°C) for 38 μm were quite linear and, for 125 μm, the linearity of the curve was adequate for the rising temperature, but it has changed for the descending one. The maximum hysteresis correspond to a 6% of capacitance at 40°C (125 μm), but for all other points, it was below 2%, exhibiting a narrow hysteresis loop; this result is supported by literature (Zhu et al., 2010). In our previous tests, it was observed the tendency of stabilization in different levels for the same temperature (rising and descending), mainly for samples thicker than 50 μm.

The influence of thickness was also observed concerning to the time necessary to stabilization of capacitance measurement under different temperatures. In general, the time of descending temperature rises, but for 38 μm there was not a great variation of time that was opposite to 125 μm, once the time quite doubled (Table III-1). Further studies must be carried out in order to understand if the behaviour in the descending temperature comes from the gelatin after T_g or from the climate chamber used in the tests.

The electrical capacitance depends on the thickness (Li et al., 2011), what can be seen in Fig.III-5. The sample with 125 μm presented a curve with a higher slope, indicating a higher sensitivity. This relationship was calculated between 40°C and 80°C according to:

$$S(m) = \Delta C / \Delta T$$

S –Sensitivity
ΔC –Quotient of the capacitance variation
ΔT –Quotient of the temperature variation

The sensitivity for the sample with 125 μm was 0.14 ± 0.010 pF/°C (sample size = 3) and with 38 μm was 0.045 ± 0.009 pF/°C (sample size = 3), being more than 3 times lower showing that higher thickness leads to higher effectiveness to distinguish the variation of temperature.

Table III-1**.** Stabilization time (in minutes) of electrical capacitance of gelatin sensor with 38 μm (868 MHz) and 125 μm (600 MHz). All data are presented as average values ± standard deviations (sample size = 3).

Temperature (°C)	**Time (minutes)**	
	38 μm	**125 μm**
40 - 60	1.8± 0.1	15.1 ± 0.5
60 - 80	2.0± 0.1	14.3± 1.6
80 - 60	2.8± 0.2	34.5± 2.7
60 - 40	2.8± 0.1	42.8± 4.2

3.4. Meat cooking application

The gelatin sensors (38 μm at 868 MHz and 125 μm at 600 MHz) were tested following the steps of meat cooking (Fig. III-6). It is clearly seen that the higher thickness (125 μm) led to a more distinguishable results mainly in the cooling steps, zone that permits the effective food safety. Considering ready-to-eat products, such as ham, sausages, it is postulated a cooling step, from 54.4 to 26.7°C, no longer than 1.5 h and from 26.7 to 4.4 °C, no longer than 5 h (USDA/FSIS, 2001), essential to reduce the activity of pathogenic microorganisms

(Mohamed, 2008). Both samples were able to show different electrical capacitances; however, with 125 μm, the system is more robust.

In the heating steps (2 up to 5), the small difference of 5°C has also resulted in a lower difference in the capacitance, what may limit the use of the gelatin sensor. Gelatin molecules have good polarization behaviour because of a large number of polar functional groups. But the presence of hydrogen bonds limits the mobility of them. To disrupt these bonds, it is necessary to improve the sensitivity indicating possible blend with other molecules (Ning et al., 2015).

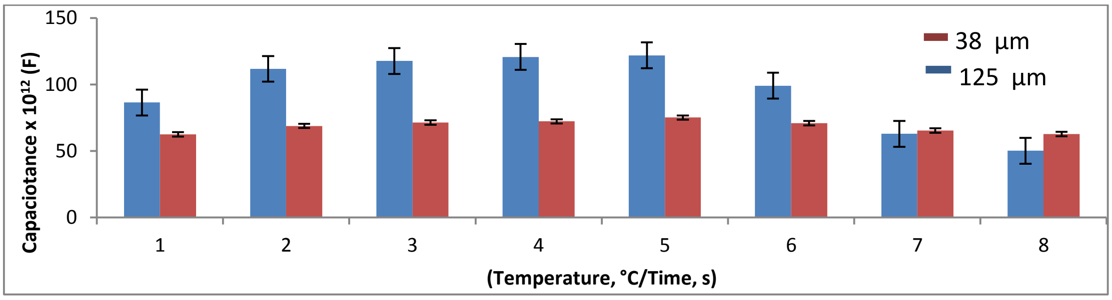


Fig. III-6. Use of gelatin sensor for monitoring the heating processing in the meat cooking: 90% RH, 125 μm (600 MHz) and 38 μm (868 MHz). Curves are: **1**: 40°C for 30 min; **2**: 65°C for 90min; **3**: 70°C for 60min; **4**: 75°C for 60min; **5**: 80°C for 60min; **6**: 80°C - 55°C for 90min; **7**: 55°C - 27°C for 120min; **8**: 27°C - 3°C for 120min. Error bar: standard deviation; n = 3.

Applying thicker samples may be the opposite of the tendency to decrease the feature size of IDC design, by employing thinner dielectric (Zhou et al., 2003). However, for the use of gelatin as a sensor, a higher thickness was essential to increase the sensitivity, but it was not possible the use at 868 MHz because of the electro-thermal breakdown.

3.4. Repeatability

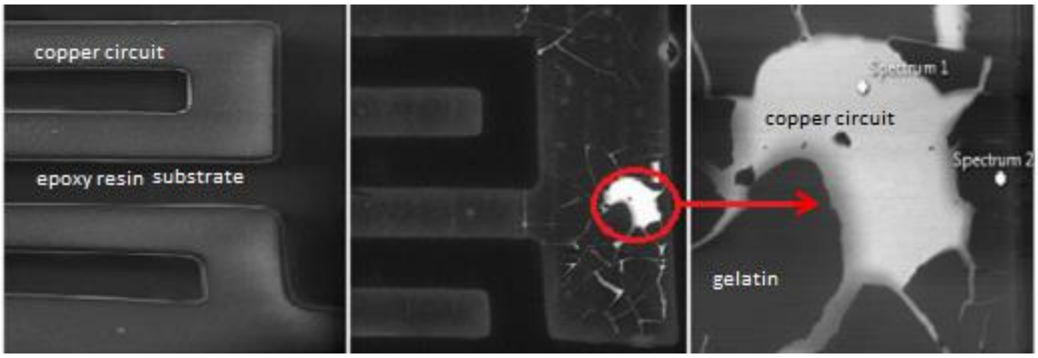
The repeatability of capacitance reading of the same gelatin sensor was investigated by using it thrice at three different temperatures (40°C, 60°C and 80°C), after storage at room temperature (around 25°C) and humidity equals to 60%, approximately. The capacitance value obtained at the first measurement was considered as the reference. In general, the capacitance reduction was around 30% and 50% for the second and third times, respectively, as shown in Table III-2. The capacitance obtained at each time and temperature was the result of the average of three measurements (repetitions) whose coefficient of variation was lower than 3%, showing a data robustness. The explanations for the reduction can be shown in Figure III-6 that shows the sensor before and after use. It can be clearly seen the loss of material (Fig. III-7a and III-7b). In the sensing region of the electrode (copper circuit), it is seen the loss of gelatin (spectrum 1) compared to spectrum 2, where there is also this material (Fig. III-7c). As the humidity used was high (90% RH), the gelatin was always wet, what facilitates the adhesion on the electrode. But, with storage in low humidity (around 60%) and ambient temperature (25ºC), the film cracks facilitating losses.

Table III-2. Percentage of electrical capacitance reduction of the same gelatin sensor with 38 μm, at temperature range 40-60-80°C, 90% RH and 868 MHz.

Temperature °C	Reduction (%)		
	First Time	Second Time	Third Time
40	0	27 ± 1.2	46 ± 1.2
60	0	32 ± 1.5	47 ± 0.9
80	0	36 ± 0.5	48 ± 0.6

The capacitance readings were stable at high humidity, situation that is reported as necessary to avoid loss of weight and consequently changes in electrical properties (Saum et al., 2000). Indeed, the most important indicator that inhibits a continuous use of the sensor is not related to electrical measurement, but to the reduction of sensitivity. After storage at low humidity, for the third time, it was 0.019 ± 0.0001 pF/°C, more than two times lower than the first time (0.045 ± 0.009 pF/°C), as shown in Table III-2.

We may conclude that it is possible to use the same biosensor in several heat treatments, as long as the humidity is kept high (above 90%). A wet storage condition could be considered (Tasca et al., 2013) or the use of a wetting additive, but these conditions can facilitate food spoilage. Thus, refrigeration should be applied. However, these are possibilities that can increase the costs and reduce the simplicity of preparing and using gelatin. Then, the best option is to use a new sensor, after finishing an essay, as it is cheap.



(**a**) (**b**) (**c**)

Fig. III-7. Images by SEM (scanning electronic microscopy) of the gelatin layer (38 µm) on the electrode: (a) electrode before use, 50X, (b) electrode after use, 50X, and (c) detail (400X) of the image from condition (b).

4. Conclusions

The use of gelatin sensor to provide accurate temperature measurements was ascertained for different thicknesses (38 µm and 125 µm) of films coated on interdigitate electrodes. For the sample with 38 µm, the system was stable for all temperatures up to 80°Cand frequency range equals to 300-900 MHz. But, for the sample with higher thickness (125 µm), the temperature induced the electro-thermal breakdown, limiting the use at 868 MHz. This phenomenon appeared around the temperature range of 60°C up to 80°C, what coincides with the T_g zone of gelatin. In order to overcome the electro-thermal breakdown, the experiments with sample with 125 µm were carried out at 600 MHz. The combination of higher thickness (125 µm) at 600 MHz in comparison with sample with 38 µm at 868 MHz resulted in a higher sensitivity and in a better condition to distinguish the different temperatures normally used in the meat cooking, mainly in the cooling steps. It points to a good trade-off between thickness and frequency, focusing to improve the electrical answers. The gelatin sensor may be used several times under the same and continuous experimental conditions (90% RH and up to 80°C) without variation in the capacitance. The reuse of the same gelatin sensor several times is not recommendable because it reduces the sensitivity as a result of mass loss after each use, when stored at low humidity. Gelatin sensors are feasible with tests under experimental conditions simulating parameters used in meat cooking. For a real production and application of the sensor, it must be considered an interaction of gelatin with compounds of the food matrix.

Part IV: Sensor-enable RFID tag

FEASIBILITY OF GELATIN SENSOR-COUPLED TO UHF RFID TAG FOR MONITORING TEMPERATURE IN MEAT COOKING

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Abstract

A passive RFID tag coated with a thin film of gelatin represents a new sensor for monitoring temperature. The temperature range applied is the one normally used during meat cooking (20°C up to 80°C) under 90% relative humidity (RH). The gelatin film is coated on three different areas (three layouts) of the RFID tag surface: all antenna; chip area; internal loop area. One tag remains uncoated to allow the evaluation of the gelatin layer impact. The frequency band used to study the RFID tag response was 700 MHz up to 1200 MHz. For the tag with all antenna coated by gelatin, there is a statistical significant difference ($p < 0.05$) at 868 MHz, 915 MHz and 960 MHz frequencies, for the different temperatures compared to the samples with other areas of coverage. In addition, for this layout, there is, approximately, a 10% hysteresis error, enabling the gelatin coated RFID tag sensor to be used for monitoring the temperature of the meat's cooking. In this way, the experiments led to the conclusion that the RFID tag coupled with gelatin film is a new device for monitoring temperature.

Keywords: temperature sensor; gelatin; RFID; read range; meat cooking.

Introduction

Thermal treatment in food industries is a common operation for bacteria destruction and enzymes responsible for food damages (Guérin et al., 2007). Temperature is usually monitored by thermocouples that are widely used because of their reliability and of being an inexpensive and robust method (Zell et al., 2009, Gillespie et al., 2016). However, as the measurement is based on spot checks and on a small number of products, it generates a large degree of uncertainty and local limited information (Wold, 2016, Guérin et al., 2007). These features have evoked new methods for temperature monitoring (Wan and Knoll, 2016).

RFID and barcode are considered as the most important data carrier devices that belong to the main category of convenience-enhancing intelligent systems (Robertson, 2012). Passive RFID technique permits that a tag assigned to each product to be read in any position without physical contact with readers (Wang et al., 2006). It is also recognized as a new generation of smart RFID tag for intelligent food packaging and notorious advantage by reduction and simplification in wiring (Badia-Melis et al., 2014, Kim et al., 2016b).

Our research group has been studying electrical properties of biopolymers to investigate how these properties depend on the temperature and humidity (Bibi et al., 2016b, Bibi et al., 2016c)

This dependence might be of interest in the field of intelligent packaging biosensor to indicate temperature and/or humidity changes featuring an innovative and unusual application of biosensor. The innovation of our studies lies on: a) use of a protein as a temperature sensor; b) proposal of using the RFID technology to monitor the temperature during processing steps and c) combination of the first and second steps.

The feasibility of using gelatin, as a temperature sensor, was already demonstrated in a previous work of this group and the efficiency of RFID technology is proven by literature (Badia-Melis et al., 2014, Kim et al., 2016b). Here, it is therefore a combination of low cost sensor based on biomaterial [10] with an Ultra High Frequency (UHF) RFID tag to monitor temperature during the meat cooking cycle. Indeed, this coupled technology brings a single identifier, and sensor information with a low cost wireless technology.

Thus, this work aims at studying the impact of gelatine layer on RFID response performance. So we have experimentally worked on how different layouts coming from different coverage areas of the RFID tag by gelatine film may influence radiofrequency (RF) sensitivity and particularly on the regulated frequencies of 868 MHz, 915 MHz and 960 MHz.

2. Material and Methods

The RFID measurements were explored considering a temperature range of 20°C to 80°C by step of 20°C and a constant relative humidity (RH) of 90%, conditions normally used in meat cooking processing. The frequency band studied was 700 MHz up to 1200 MHz, and then only we extract the results from the three frequencies already cited. The variable of response adopted was the Theoretical Read Range (TRR).

2.1. Preparing samples

Gelatin was used (Merk, Darmstadt, Germany) with a concentration 10% w/v (Fakhoury et al., 2012). The bubbles dissolved in the solutions were removed by vacuum conditions. The solution (250µl) was coated onto the surface of commercialized UHF passive RFID tags (Tageos Company, Montpellier, France), using an E409 blade coater from Erichsen (Hemer, Germany). The coater was equipped with the number 4 blade having spires of 0.51 mm, in order to create a humid film deposit having a thickness around 2 µm. The speed was set to 1 mm/s. The sample was left to dry for 24 hours at room temperature and at 50% of relative humidity. The coverage areas are shown in Fig. IV-1: (a) layout 1 (all antenna area); (b) layout 2 (chip area); (c) layout 3 (internal loop area); layout 4 (uncoated).

For all tests, passive RFID tags were used in order to reduce costs. These tags are composed of an antenna and a RFID chip. Moreover, they are battery-free which makes their lifetime long and cost negligible, contrary to active or semi passive ones that use a battery (Papapostolou and Chaouchi, 2011).

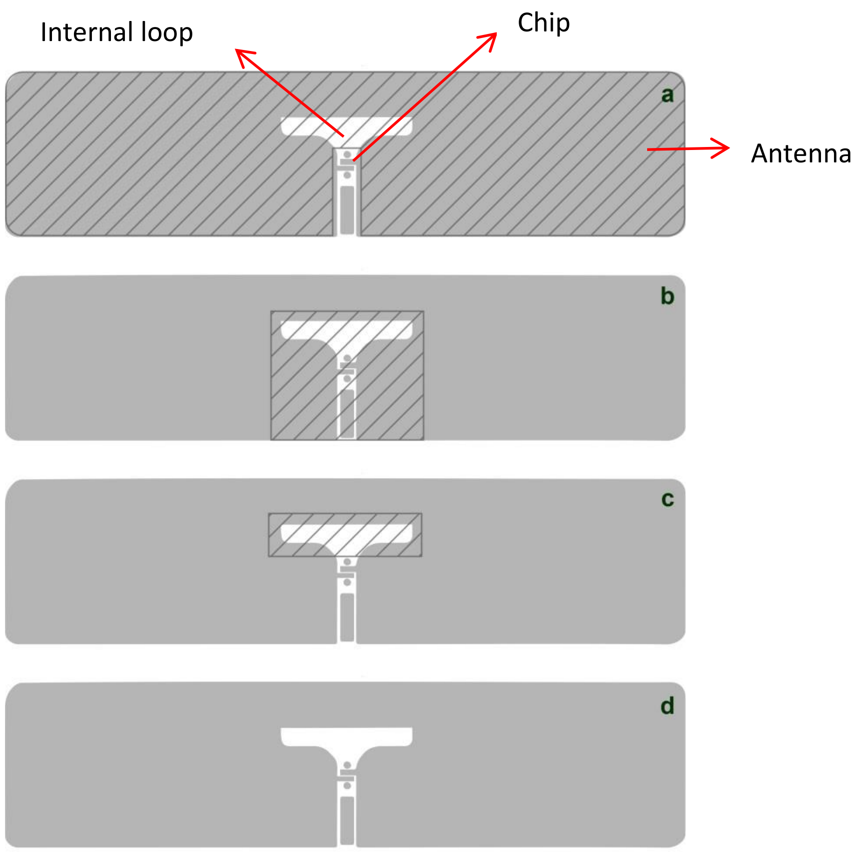


Fig.IV-1. Gelatin sensor-enable RFID tags with different coverage areas: layouts 1 (a), 2 (b), 3 (c) and 4 - uncoated (d). The thickness of the gelatin layer was equal to 1.8 μm.

2.2. Thickness

The thickness of the layer deposited onto RFID tag was measured at room temperature and humidity with a profilometer Dektak resolution at a maximum scan length of 0.033 μm (Bruker, USA). The thickness of the gelatin layer was equal to 1.8 μm for the tags used in layouts 1, 2 and 3, respectively.

2.3. RFID performance

The performance of RFID tags was evaluated by the Tagformance of Voyantic Company (Espoo, Finland) which is a directional coupler 700-1200 MHz (Voyantic Tagformance^{TM} Lite: http://www.voyantic.com). The Tagformance is linked on a side by a near field antenna with a RF cable and on the other side to the Tagformance measurement software to record the measurements. The RFID tags were positioned onto the Tagformance's near field antenna (snoop pro antenna from voyantic), inside of a climatic chamber (Espec, Japan) to control both humidity and temperature (Fig. IV-2).

From the Tagformance, the measurements were based on an electromagnetic threshold technique, in which the frequency was changed from 700 MHz to 1200 MHz in a step of 1 MHz. At each frequency, the transmitted power was increased by 0.1 dB up to the tag to be activated and to respond properly. The minimum transmitted power to activate the tag was measured at each frequency. This is also possible to have Theoretical Read Range (TRR) calculated with help of Friis Equation (Dobkin, 2005). Thereafter, we will take a relative differential measurement from TRR (TRR(%)) defined in equation (1).

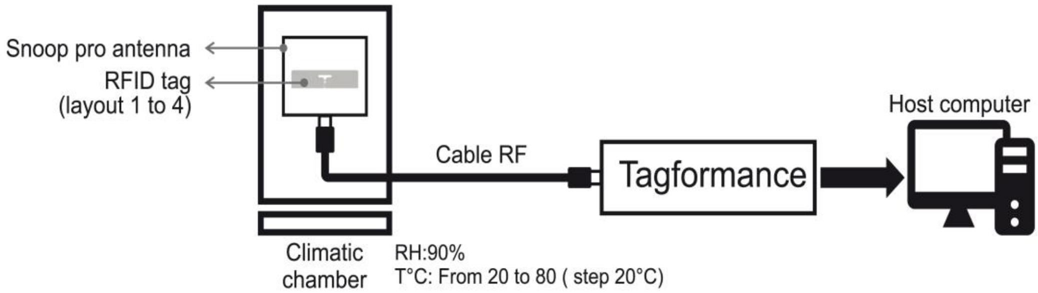


Fig. IV-2. Experimental set-up used for the RFID tests.

The temperatures used were 20°C, 40°C, 60°C and 80°C. After stabilization of humidity (90% RH) , the measurements were made under a frequency band of 700 MHz up to 1200 MHz, at each temperature, starting at 20°C by step of 20°C. After reaching 80°C, measurements were taken decreasing temperatures (60°C, 40°C and 20°C). To analyse the performance of the RFID tags, UHF frequency band was chosen (860 MHz to 960 MHz), as defined in ISO/IEC 18000 standardization documents (Santos et al., 2014), focusing 868 MHz, 915 MHz and 960 MHz. The results were presented as absolute value of relative variation of the TRR (%) and sensitivity.

$$|TRR(X) - TRRup(20^{\circ}\ C)| \times \frac{100}{TRRup(20^{\circ}\ C)} \text{ (for each frequency)} \qquad (1)$$

TRR(x) - theoretical read range at certain temperature (20°C, 40°C, 60°C and 80°C)
TRRup (20°C) - theoretical read range at 20°C at the beginning
100 - factor to express the results in percentage (%)

$$S(\%/°C)) = \frac{\Delta\, TRR(\%)}{\Delta T\ (°C)} \quad (2)$$

S (%/°C) – sensitivity on measurement interval
ΔTRR (%) – difference TRR (%) value on measurement interval
ΔT (°C) – temperature difference on measurement interval

2.4. Statistical Analyses

Each experiment was made thrice and for all statistical analyses, it was used a significant level of 5% for Fisher's test and Statistica software, for Windows, version 13.0 (Tulsa, USA). All data are presented as average values $\pm$ 1 standard deviations. The following analyses were carried out:

1- Layout that permitted a significant difference among 868 MHz, 915 MHz and 960 MHz for the same temperature.
2- Layout that permitted a significant difference among pairs of temperatures (combination of 20°C, 40°C, 60°C and 80°C) at 868 MHz, 915 MHz and 960 MHz.
3- Frequency in which the same value of TRR was observed at the same rising and descending temperature.

3. Results and discussion

The RFID system can be operated in several frequency bands, but the most used is the Ultra High Frequency (UHF), specifically the frequencies managed by regulations of individual countries: 868 MHz (Europe) and 915 MHz (United States) (Sanghera, 2007). At UHF, there are many advantages, such as: transfer data faster than low and high-frequencies (Ruiz-Garcia and Lunadei, 2011), longer communication distance, higher data rates, as well as smaller antenna size in RFID systems (Sun et al., 2010). However, this lack of standardized frequency is hampering the implementation of RFID technology for different applications (Sanghera, 2007). It is reported in the literature that as 915 MHz and 868 MHz are close frequencies, the propagation characteristics and conclusions can be also extended to each other (Angle et al., 2014). This approach is not totally applicable because there was a significant difference for the layouts 1 and 2 (p-level < 0.05) between the aforementioned frequencies, indicating different behaviour.

Fisher's test, considering 5% of significance level, showed that temperature, layout, frequency and their interaction effects influenced significantly the radio frequency answer whose values for the four layouts are presented in Fig. IV3-6. Comparing the results of the three layouts (Fig IV3-5) with the reference layout (Fig. IV-6), it is clear there is an influence of the gelatin on the Sensor-RFID response. However, the better performance of the layout 1 in terms of absolute value of relative variation was outstanding, confirming the importance of the whole coverage of the antenna as the layout suitable for monitoring the temperature.

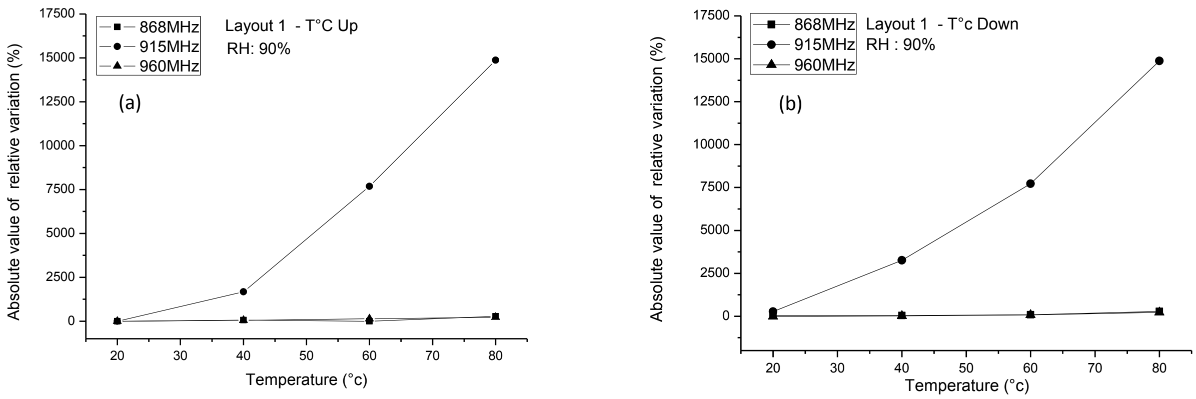


Fig. IV-3. Curves of absolute value of relative variation (%) of the Theoretical Read Range (TRR) versus temperature (°C) for: (a) rising temperatures (20°C, 40°C, 60°C and 80°C) and (b) decreasing temperatures (80°C, 60°C, 40°C and 20°C): Layout 1. Experiments made in triplicate with coefficient of variation below 10%.

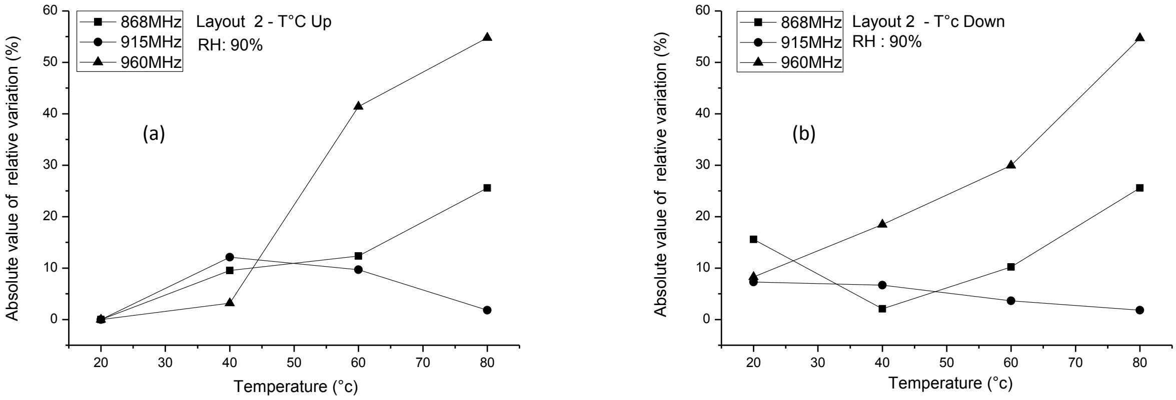


Fig. IV-4. Curves of absolute value of relative variation (%) of the Theoretical Read Range (TRR) versus temperature (°C) for: (a) rising temperatures (20°C, 40°C, 60°C and 80°C) and (b) decreasing temperatures (80°C, 60°C, 40°C and 20°C): Layout 2. Experiments made in triplicate with coefficient of variation below 10%.

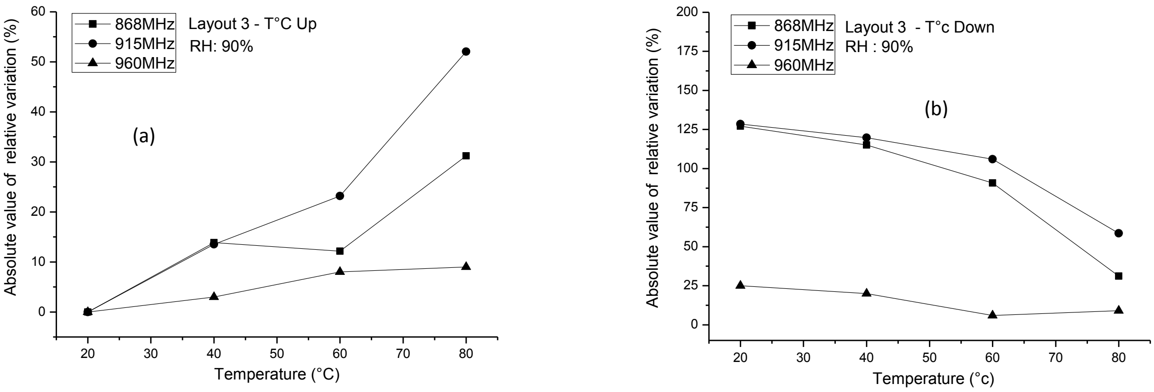


Fig. IV-5. Curves of absolute value of relative variation (%) of the Theoretical Read Range (TRR) versus temperature (°C) for: (a) rising temperatures (20°C, 40°C, 60°C and 80°C) and (b) decreasing temperatures (80°C, 60°C, 40°C and 20°C): Layout 3. Experiments made in triplicate with coefficient of variation below 10%. The scale of the Y-axis was different due to the difference in behavior of the curves: rising and decreasing temperature.

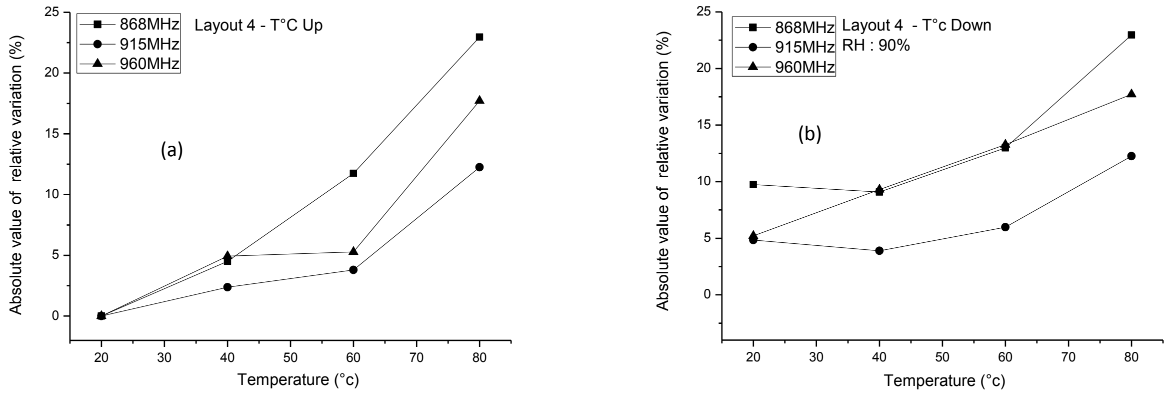


Fig. IV-6. Curves of absolute value of relative variation (%) of the Theoretical Read Range (TRR) versus temperature (°C) for: (a) rising temperatures (20°C, 40°C, 60°C and 80°C) and (b) decreasing temperatures (80°C, 60°C, 40°C and 20°C): Layout 4. Experiments made in triplicate with coefficient of variation below 10%.

The TRR is a result of a given temperature and a correlation between them may be established; it is desirable that the TRR value for rising temperature would be the same for descending temperature, implying then no hysteresis. In layout 1 at 915 MHz this condition was fulfilled at a critical temperature zone that is necessary for the effective control of pathogens such as *Clostridium perfringens* (60°C up to 80°C and 80°C up to 20°C) (Fig. IV-7). Even though in layouts 2 and 3 at 915 MHz and 960 MHz the absence of hysteresis was observed, there is no significant difference among the different temperatures (20°C, 40°C, 60°C and 80°C); thus, they are not suitable for monitoring the temperature at 915 MHz and 960 MHz.

Besides the behaviour on the hysteresis, the sensitivity at 915 MHz was also remarkable comparing to the others frequencies (868 MHz and 960 MHz); it may be seen by the inclination of the curves (Fig. IV-3). The hysteresis error was 28% and 31% for 868 MHz and 960 MHz, respectively; these values are around 3 times higher compared to 915 MHz those which was at 10% at 40°C that is inside the acceptable band of variation. Further, the sensitivity was influenced by the temperature band and also the rising (up) and decreasing temperature (down) and by this variable it can be seen also the outstanding results at 915 MHz (Table IV-1).

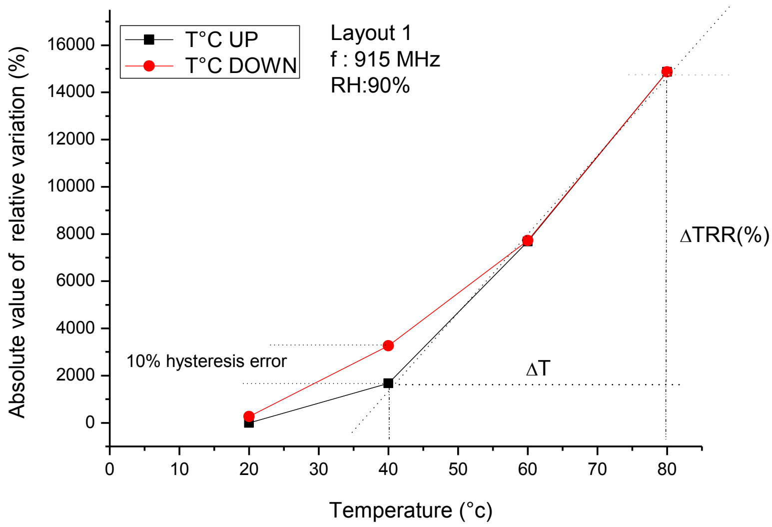


Fig. IV-7. Hysteresis at 915 MHz at rising temperature (20°C, 40°C, 60°C and 80°C) and decreasing temperature (80°C, 60°C, 40°C and 20°C): layout 1. Experiments made in triplicate with coefficient of variation below 10%.

Table IV-1. Sensitivity of gelatin (%/°C) at rising (up) temperatures (20-40°C and 40-80°C) and decreasing (down) temperatures (80- 60°C and 60-20°C): 868 MHz, 915 MHz and 960 MHz at 90% HR: layout 1.

Sensitivity (%/°C)

Freq (MHz)	**Temperature UP (°C)**		**Temperature DOWN (°C)**	
	20-40°C	40-80°C	80- 60°C	60-20°C
868	0.025	14	10	1
915	83	330	330	186
960	3.75	3.75	5	1.75

The frequencies normally used in UHF RFID system operate with reduced readability near loads of perishable products with high-water content. Water absorbs radio frequency energy, decreasing the read range (Amador and Emond, 2010). Taking as reference the normal value of read range for passive tag at 860-960 MHz, that is below 10 m (Plos and Maierhofer, 2013). In all layouts the TRR values were inside this limit showing trustworthiness. However, the influence of the temperature is observed at 80°C, once at this value the TRR was above 10 m.

The influence of water may be considered as a key noise parameter (KNP), as it reduces the read range. The knowledge of KNP is mandatory in systems based on electromagnetic waves, such as RFID. In our previous studies (to be published), essays were carried out under humidity of 40% and 90% RH and the influence of water on the TRR changes markedly as function of humidity and frequency. For 840 MHz, the TRR variation was around 90% for 20°C and up to 130% for 60°C. For 868 MHz the variation was around 225%. However, considering 80°C for both frequencies, the variation of TRR was around 90% and 100% (840 MHz and 868 MHz, respectively). This lower TRR variation compared to 60°C may be related to an influence of the gelatin glass transition (Tg) (Boltshauser et al., 1991, Story et al., 1995). Thus, beside water influence, there is also an influence of Tg on the TRR. Herein, both water and Tg did not preclude the sensitivity in all three layouts, showing the robustness of this new sensor to overcome these KNPs.

Based on 868 MHz, 915 MHz and 960 MHz, it may be concluded that layout 1, compared to layout 2, was superior once there was a significant difference in TRR values at the critical temperature zone: heating (60°C up to 80°C) and cooling (80°C up to 20°C) for all frequencies. Thus, it confers flexibility to attend the different regulations of the countries regarding to which frequency, 868 MHz, 915 MHz or 960 MHz, is adopted.

For the regions where 868 MHz is used, layout 2 may be adopted but, at this frequency, layout 1 is more suitable to be used as it permits to distinguish better the difference of TRR values among the temperatures. It is not possible to use layout 3 for all frequencies (868 MHz, 915 MHz and 960 MHz), because there was not a significant difference in TRR values among the temperatures.

These results show that the way the gelatin was coated onto the tag (Fig. IV-1) clearly influences the TRR value. Based on layout 2, whose difference from the layout 3 was the coverage of the chip area, it may be inferred that it was the explanation for better results. However, the coverage of antenna in layout 1 (without the chip area being covered as well) was the key feature for the temperature sensor. As the antenna transmits information, it is reasonable to restrict a contact with the sensing material (gelatin).

4. Conclusions

Passive RFID tag coated by a gelatin thin film was presented as a wireless monitoring temperature sensor for meat cooking application. Indeed, we have demonstrated for high relative humidity (90%) and meat cooking cycle, that gelatine layer coupled on commercial UHF RFID design tag follows the temperature variation cycle when there is an RFID reading. For excellent sensitivity and lower hysteresis error, the gelatin layer must cover all the antenna area (Layout 1). This sample has been compared to the reference sample (uncoated), and two others samples partially coated, we have shown that gelatine layer impact with temperature, but also with RFID antenna impedance. The electric properties of gelatine layer varies with temperature, this induces electromagnetic change in RFID response. We obtain better results for 915 MHz with an error hysteresis of 10% and a sensitivity of twenty twice important than the others frequencies (868 MHz and 960MHz). Moreover, the layout 1 at 915 MHz, points to the potential use of this new sensor for heating and cooling steps during meat cooking; it shows then robustness of the gelatin sensor-enable RFID. The present results are encouraging and the perspectives are to control and optimize the gelatin layer as well as develop prototype for future testing in real conditions of meat cooking.

Part V: General discussion

This thesis presents an original research based on the electrical properties of biopolymers to investigate how these properties depend on temperature and humidity. The literature is relatively scarce concerning protein based material, which is more restrict when considering variation with temperature and/or humidity on a large frequency range.

Our research line was based on the temperature dependence of biopolymers assuming its interest in the field of intelligent packaging sensor to monitor temperature, thus featuring an innovative and unusual application of biosensor that is normally applied to control food quality markers.

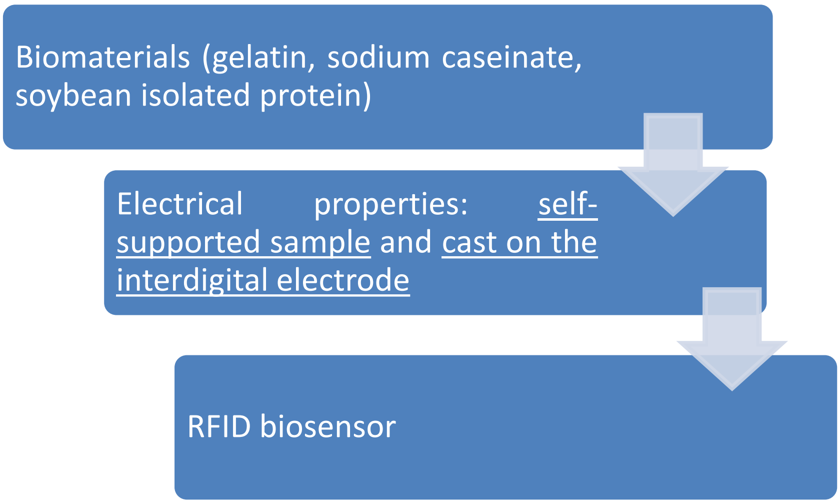
Thermal treatment is the most used method focusing destruction of pathogenic microorganisms. The effectiveness of its control results in food safety. Its current control is made by thermocouples, but this method is based on spot checks (contact or invasive sensor) of a small number of products generating a high degree of uncertainty and local limited information. These features have evoked new methods for temperature monitoring focusing mainly to non-contact (non-invasive) temperature measurements.

The proposal of using a biomaterial as a temperature sensor was the first part of the project. The second one was to couple it with RFID tag. For both parts, there are no publications. Moreover, the tests made in this project considered an application in meat cooking; thus, it is another innovative feature once RFID is not used to monitor temperature in processing steps but only in the cold channel.

In this thesis, we have faced with the innovative proposal of using RFID coupled with a biomaterial as a sensor of temperature. This project may be considered as the first step for a new concept of sensor whose innovation was based on three different pillars:

1- Use of RFID technology as a tool to monitor processing steps;
2- Use of proteins as a sensor of temperature;
3- Proposal of gelatin sensor-enable RFID tag as a temperature sensor coupling the already known advantages of RFID and a new class of sensor based on biomaterial.

The intention of this project was to propose some device useful and easily applicable to many goals. First, it was necessary to define the requirements in sensing criteria/conditions and their critical values. It was applied the environmental variables of temperature (20°C up to 80°C) and humidity (90% HR) normally used in the meat cooking. In a second step, the sensing biomaterial was developed upon screening electrical properties in the critical conditions identified previously. The third step deals with the coupling of sensing material with RFID tag. The planning is sum up in the following scheme:



The biopolymers proposed in this thesis, gelatin, sodium caseinate and soybean isolated protein, were chosen based on previous experience of Agropolymer Engineering and Emerging Technologies IATE. Besides them, both chitosan and pectin were tested, but in the screening tests, the performance to form the film was not suitable because of the low concentration achieved.

All the biopolymers were temperature dependent, but only at high humidity (90% RH) there was a significant difference among the temperatures. However, the gelatin has shown always higher sensitivity and it was chosen to be used in the following steps. The tests made at lower humidities (20 and 55% HR) have also shown sensitivity of the biopolymers, but without statistical difference among the temperatures. These facts point the importance of the water polarization in the functioning of the temperature sensor based on biopolymers (Fig. II-3). The influence of the water in the results was shown by the tests using both self-supported sample and cast on the interdigital electrode techniques.

This feature limits the use of this sensor only at high humidity environments that coincidently is the same used during meat cooking (90-95 % HR), process that was chosen as reference in this thesis.

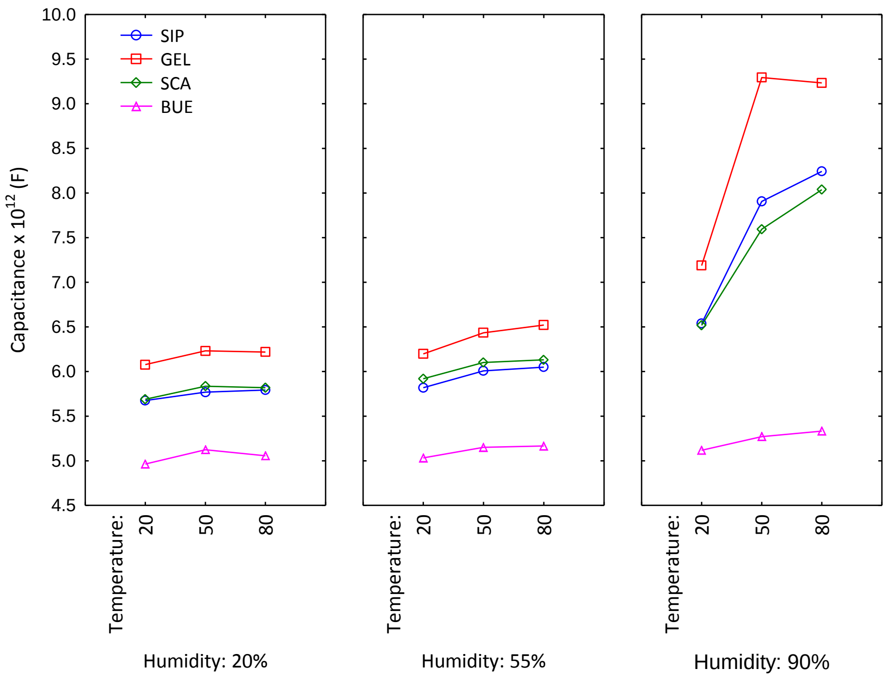


Fig. II-3. Influence of temperature (20°C, 50°C and 90°C) and humidity (20%, 55% and 90%) on the capacitance for soybean isolated protein (SIP), gelatin (GEL) and sodium caseinate (SCA) and blank uncoated (BUE), at a frequency of 868 MHz. All coefficients of variation were lower than 10%.

We have started the studies to improve the sensitivity. The criteria were not to add any other component in order to avoid possible changes in the electric properties of gelatin and also to remove the simplicity of using a single sensing material. The thickness was chosen as a factor to induce rising of the sensitivity.

The results have shown that the sensitivity is thickness dependent, but because of the thermo-electrical breakdown, a balance between thickness and frequency should be stablished. In the tests made with sample at 38 μm, the electrical capacitance was normally read at 868 MHz (frequency taken as reference) at all temperature band (20°C up to 80°C) but with the sample with 125 μm, at same frequency, the slope capacitance versus temperature curve decreased between 60-80°C. The gelatin DSC analyses have shown that there is a Tg in this band causing this phenomenon; this behaviour is supported by the literature.

The way applied to overcome this phenomenon was to reduce the frequency. Based on the permittivity studies, the frequency of 600 MHz was chosen to work with sample of 125 μm. The sensitivity for the sample 125 μm at 600 MHz was 0.14 pF/°C, value three times higher than 38 μm at 868 MHz (0.045 pF/°C). It shows that higher thickness leads to higher effectiveness to distinguish the variation of temperature.

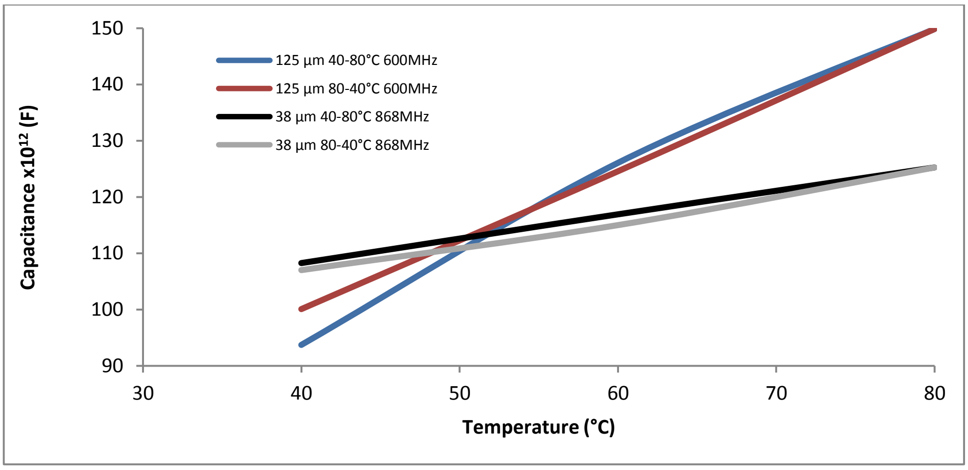


Fig. III-4. Hysteresis of gelatin from 40°C to 80°C and 90% RH for two thicknesses: 125 μm (600 MHz) and 38 μm (868 MHz). Experiments made in triplicate with coefficient of variation below 10%.

The last step was to evaluate the behaviour of the gelatin coupled with RFID tag. The environmental conditions was constant relative humidity (90% HR) and with variation of temperature (20-40-60-80°C). The frequency band applied was from 700 up to 1200 MHz. These variables were used at three different layouts (Fig. IV-1).

The variable of response was the Theoretical Read Range (TRR) that has shown dependence for temperature, frequency and layout. This feature has shown the feasibility of this new sensor. The layout 1 (coverage of whole antenna by gelatin film) has delivered better results. For both regulated frequencies, 868 and 915 MHz, there was a significant difference among the temperatures in the critical zone, related to microbiological control (60-80°C and 80-20°C). Moreover, in layout 1 at 915 MHz, there was no hysteresis, pointing the potential use of this new sensor for heating and cooling steps during meat cooking; it shows then robustness of the gelatin sensor-enable RFID (Fig. IV-3).

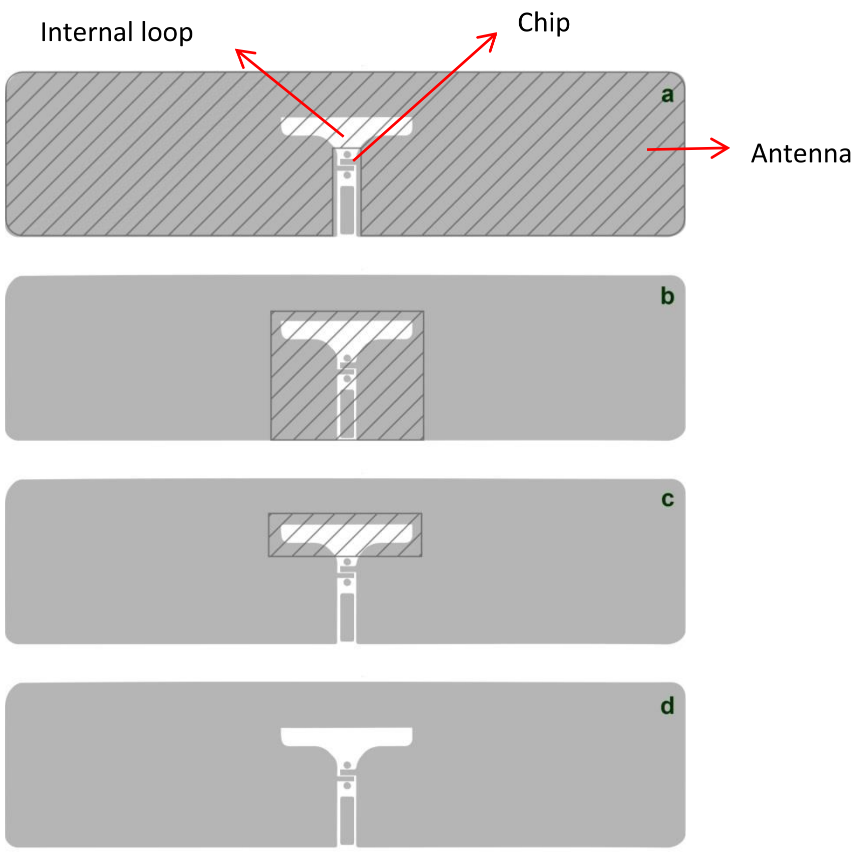


Fig.IV-1. Gelatin sensor-enable RFID tags with different coverage areas: layouts 1 (a), 2 (b) and 3 (c) and 4 - blanch uncoated (d).

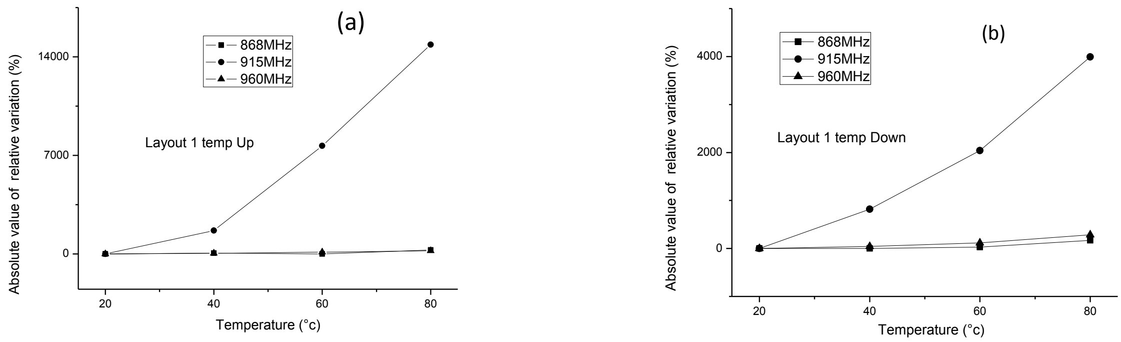


Fig. IV-3. Curves of Theoretical Read Range (TRR) versus frequency (MHz) for rising temperature (20°C, 40°C, 60°C and 80°C) and for decreasing temperature (80°C, 60°C, 40°C and 20°C): Layout 1.

Conclusions and perspectives

The use of RFID tag is not new; a sensor based on biopolymers is an innovation. The core idea was to match this preeminent technology with sensing material coming from renewable sources.

Our project has born on considerable challenge by proposing so new and unusual application of a biosensor: monitoring temperature. We have got success in our findings and to continue the development, the next step is to evaluate its use in real conditions of meat cooking. The following challenges are foreseen:

1- How to overcome the metal shield that interferes in the electromagnetic waves used by RFID technology? At the same way, there is an influence of water, but it was demonstrated herein that this key noise parameter did not disturb sufficiently to supplant the use of the RFID. The reasonable options are to use the readers inside the oven or the use of this technology with a probe (similar to what was made in part 4).
2- The characterization of gelatin was done by addressing the capacitance. Other electrical properties should be studied such as permittivity and conductivity.
3- To establish a protocol of how to use the RFID tag concerning to aspects such as location and position of the readers.
4- In order to reinforce the effectiveness of this new temperature sensor, studies should be made in order to compare it with traditional thermocouples.

Finally with this new proposal of temperature sensor, we can face two more exciting projects:

1- Development of a wireless thermocouple based on RFID technology and biomaterial as a sensing component;
2- Exploration of how to integrate this new temperature sensor into the food quality program. It is reported, by literature, the biocompatibility characteristics of gelatin with several quality markers (NH_2, COOH, $CONH_2$, OH and SH) because of different interactions (H-bonding, hydrophobic interactions, covalent, etc.). It is reasonable to consider the use of the same sensor for both control of temperature and food spoilage.

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Annexes

Annex 1 – Design matrix for the 3^2 factorial design for the capacitance

	Coded variables		Original variables	
Experiments	**Humidity**	**Temperature**	**Humidity**	**Temperature**
1	-1	-1	20	20
2	-1	0	20	50
3	-1	+1	20	80
4	0	-1	55	20
5	0	0	55	50
6	0	+1	55	80
7	+1	-1	90	20
8	+1	0	90	50
9	+1	+1	90	80

Annex 2 – Experimental design for the capacitance measurements.

	Protein	**Humidity (%)**	**Temperature (°C)**
1	soybean isolated protein	20	20
2	soybean isolated protein	20	50
3	soybean isolated protein	20	80
4	soybean isolated protein	55	20
5	soybean isolated protein	55	50
6	soybean isolated protein	55	80
7	soybean isolated protein	90	20
8	soybean isolated protein	90	50
9	soybean isolated protein	90	80
10	gelatin	20	20
11	gelatin	20	50
12	gelatin	20	80
13	gelatin	55	20
14	gelatin	55	50
15	gelatin	55	80
16	gelatin	90	20
17	gelatin	90	50
18	gelatin	90	80
19	casein	20	20
20	casein	20	50
21	casein	20	80
22	casein	55	20
23	casein	55	50
24	casein	55	80
25	casein	90	20
26	casein	90	50
27	casein	90	80
28	uncoated	20	20
29	uncoated	20	50
30	uncoated	20	80
31	uncoated	55	20
32	uncoated	55	50
33	uncoated	55	80
34	uncoated	90	20
35	uncoated	90	50
36	uncoated	90	80