DETERMINING THE QUALITY OF PASTEURISED AND HOMOGENISED COW MILK USING MICROWAVE SENSORS

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DEDICATION

I would like to dedicate this PhD thesis to my parents, Mrs. Ashadevi Joshi and Mr. Himanshubhai Joshi, without whose continued motivation, sustained support, and everlasting belief in me, this passionate dream could not have been a reality.

ABSTRACT

More than 6 billion people worldwide consume milk and milk products and this number is rapidly growing every year (FAO, 2015a), there are numerous occurrences where the milk quality was below acceptable standards causing severe health hazards among consumers including young children. The aim of this research work is to design and develop a novel, microwave spectroscopy, approach for determination of overall quality of milk. In particular, this quality determination of milk products was achieved by identification of deterioration or spoilage of milk over time, classification of the milk product based on composition (e.g. fat content), in addition to the contamination (e.g. adulteration due to presence of detergents, urea). An extensive literature review was carried out to establish the scope of the PhD work and in order to achieve the objectives. Current advancements were studied along with the traditional methods of milk quality testing to identify the key areas where further development can take place to enable the quality control of milk products outside the laboratory premises. This work addresses the drawbacks in currently employed methodologies and attempts to overcome or minimize their overall limiting effect.

The application of this sensor system is aimed within the milk supply-chain hierarchy after the production at dairy plants and before sale to allow easy and real-time quality testing. The dielectric property tests were conducted to produce unique spectral signatures for three mainly consumed categories of fresh milk; whole milk, semi-skimmed milk and skimmed milk bought from a supermarket over a period of a week, which served to build a reference database. Based on these spectral signatures for the three categories of milk, a planar, microwave resonator sensor acting as a fluidic sensor was designed, simulated and fabricated to determine spoilage, classification of milk and identify presence of contamination. This work has achieved distinct results to verify the statement, followed by validation, to serve as a platform for the establishment of a laboratory based prototype model to test overall quality of Milk products, with coefficient of determination $R^2 \geq 0.95$ in all experimental measurements.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

This chapter presents the foundation of this research, introducing the fundamental concepts and global trends with respect to milk products and their quality detection and control, giving a general idea of the problem and hence emphasizing the need for this project. Furthermore, the aim and objectives of the research are laid out in this chapter following a discussion based on facts and figures in the dairy sector around the world, the challenges involved and a brief idea of drawbacks in current techniques, with further critical analysis being made in Chapter 2 of the thesis.

1.1 Milk and Global Dairy Sector

Milk and dairy products play an important part not only in the diets we follow daily, but also in the overall economy of many developing as well as developed nations. Milk is a single food source of good quality nutrients like proteins, carbohydrates, fat, minerals and vitamins in a significant amount compared to any other single food (Afzal *et al.*, 2011). Milk and dairy products are nutritiously rich foods due to having the essential nutrients in appropriate amounts. Besides being a source of a balanced diet, detailed analyses have shown that there are unique and very complex structures present in milk structure that contribute towards various health-promoting functions (Cifelli, German and O'Donnell, 2011).

Table 1.1 Milk composition for different mammal species

Milk From Mammals	Fat Content (Lipids) %	Protein Content %	Carbohydrate (Lactose) %	Ash Content %	Total Milk Solids %
Human	3.8	1.0	7.0	0.2	12.2
Cow	3.7	3.4	4.8	0.7	12.7
Buffalo	7.4	3.8	4.8	0.8	16.8
Goat	4.8	2.9	4.1	0.8	12.3
Sheep	7.4	4.5	4.8	1.0	19.3
Camel	5.4	3.8	5.2	0.7	15.0

Table 1.1 highlights some of the main components in selected mammal species as discussed by Fox, (2011b). The dairy sector is the fastest growing industry, specifically in Asian, Latin American and Caribbean parts of the globe with the increasing consumption of milk products in recent decades (Gerosa and Skoet, 2013). The use of milk products is regularly growing around the world pertaining to several factors, like, improving nutritional awareness, changing dietary patterns, increasing incomes and population as well as urbanisation and overall development in the economies (Ministry of Agriculture - Republic of Kenya, 2011), whereas the developed nations already had a higher consumption of milk products per capita (FAO, 2015a). The increasing intake of dairy products provides vital health benefits to a large population of the world, except the fact that millions of people in developing nations still cannot afford this better quality diet which is expensive due to the inherently high costs of milk production or its subsequent stage of milk processing (Kenny, 2013).

More than 6 billion people worldwide consume milk and milk products, most of which belong to the developing nations of the world, and this number is rapidly growing (FAO, 2015a). This growth can be further seen from Figure 1.1, which highlights the fact that the global milk production has drastically increased by 54%, which is, from 482 million tons in 1982 to 801 million tons in 2015. This growth in 33 years, is projected to further increase by around 175 million tons (23%) by the year 2024 with comparison to that of the base years (2012-14) (FAO, 2015c) (OECD and FAO, 2015).

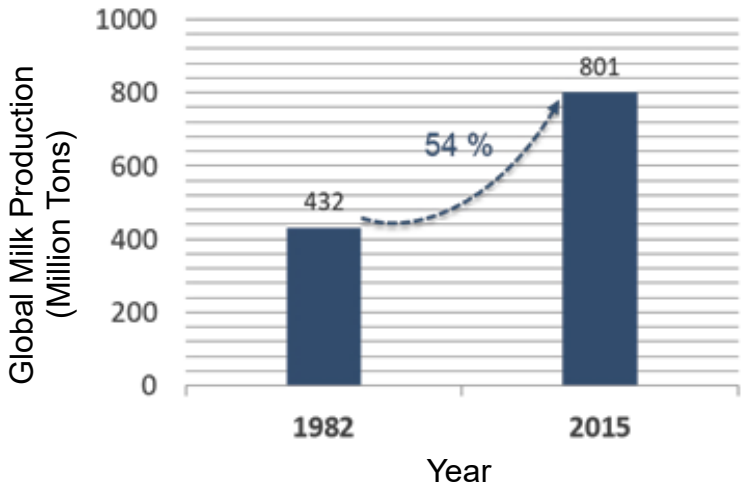

Figure 1.1 Global Growth of Milk Production

These numbers show how milk and milk products are an integral part of the daily food, especially for those who follow a vegetarian diet due to their nutritious values. The World Health Organisation (WHO) reports that around 600 million people in the world, i.e. approximately 1 in every 10 people, fall ill after eating contaminated food and about 420,000 of them die every year (WHO, 2017).

Moreover, raw and unpasteurised milk are one of the main reasons for foodborne bacteria such as Campylobacter and Enterohaemorrhagic Escherichia coli, respectively, affecting millions of people globally besides other factors like raw or undercooked poultry, drinking water and undercooked meat, fresh fruits as well as vegetables. This fact signifies the importance of processing raw milk.

Figure 1.2 shows the very complex milk composition with almost 400 fatty acids, 18 different amino acids, several minerals, vitamins and other solids. Mainly it comprises of water as fundamental element. This composition of milk collectively makes it a very nutritional platform not only for the consumers but also for the bacteria to develop and breed. Due to this reason,

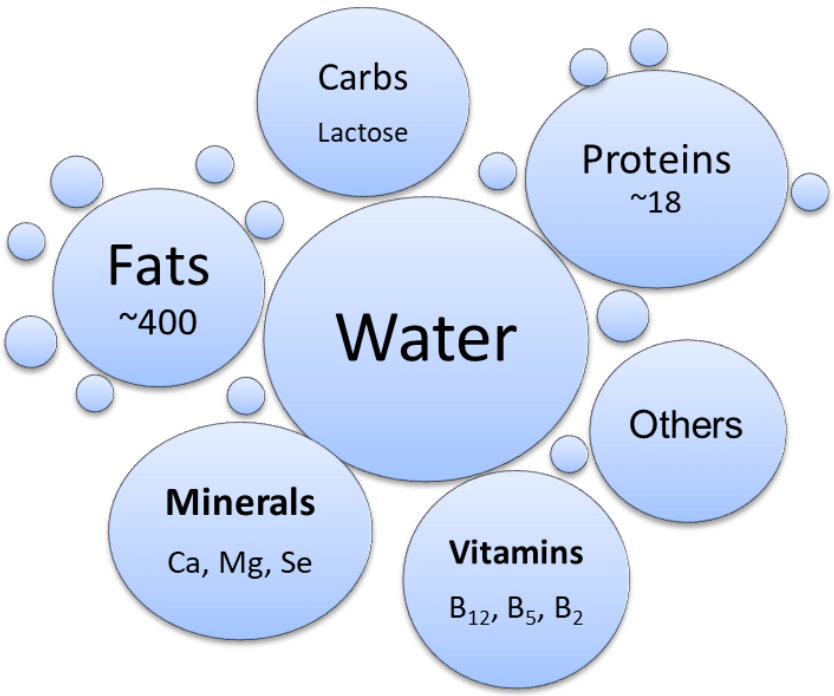

Figure 1.2 The complexity of milk composition

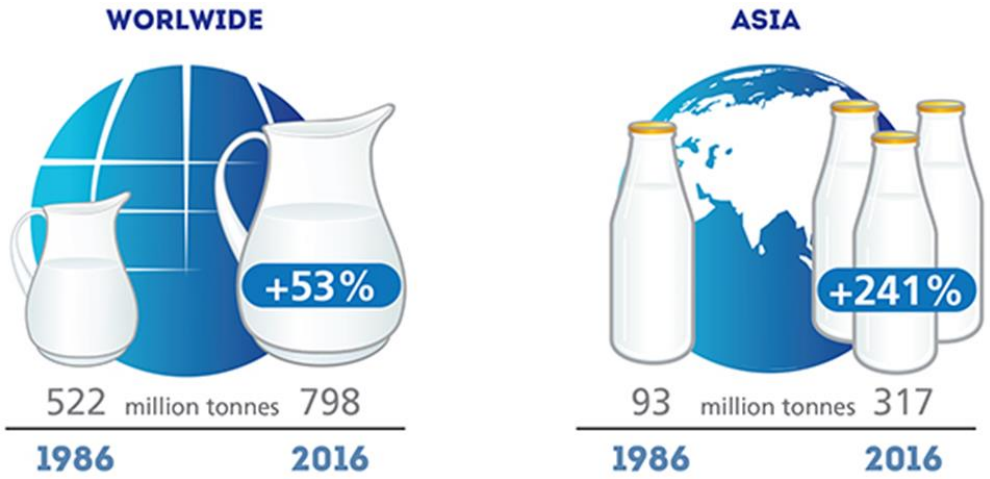

Figure 1.3 The global growth of Milk Production around the world

(Source: http://www.fao.org/resources/infographics/infographics-details/en/c/273893/ ©: FAO**)**

Figure 1.3 illustrates the global growth of milk production emphasizing on the fact that Asian dairy sector has grown by 241%. Figure 1.4 shows major 10 dominant countries producing milk and covering a share of 62% milk production worldwide, defining the major areas in the world with India, United States of America, China, and Pakistan sharing 42% of overall global milk production. This also refers to the size of the problem these countries encounter due to having a large and globally dominant dairy sector.

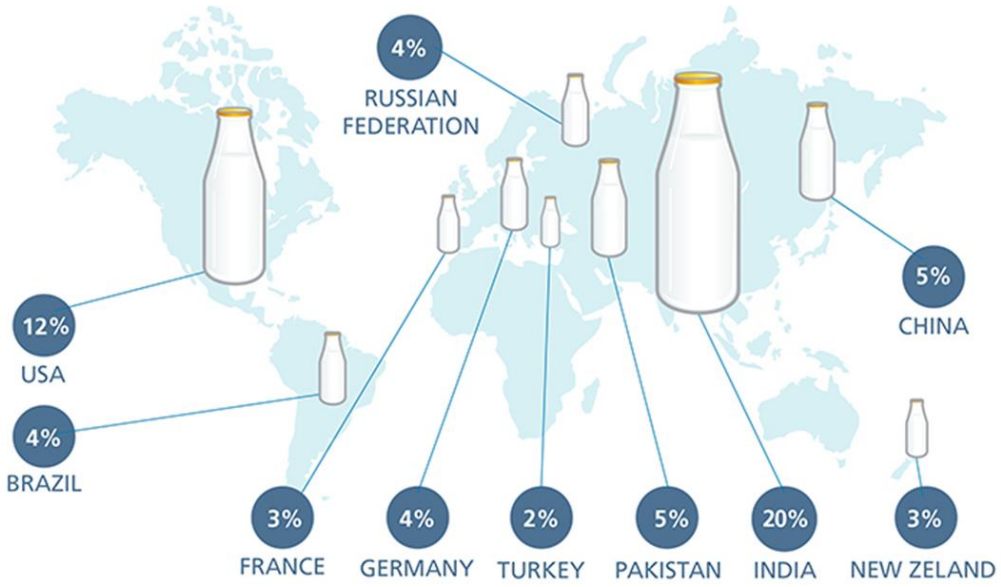

Figure 1.4 The top 10 milk producing countries contributing to 62% of the global Milk Production

(Source: http://www.fao.org/resources/infographics/infographics-details/en/c/273893/ ©:FAO**)**

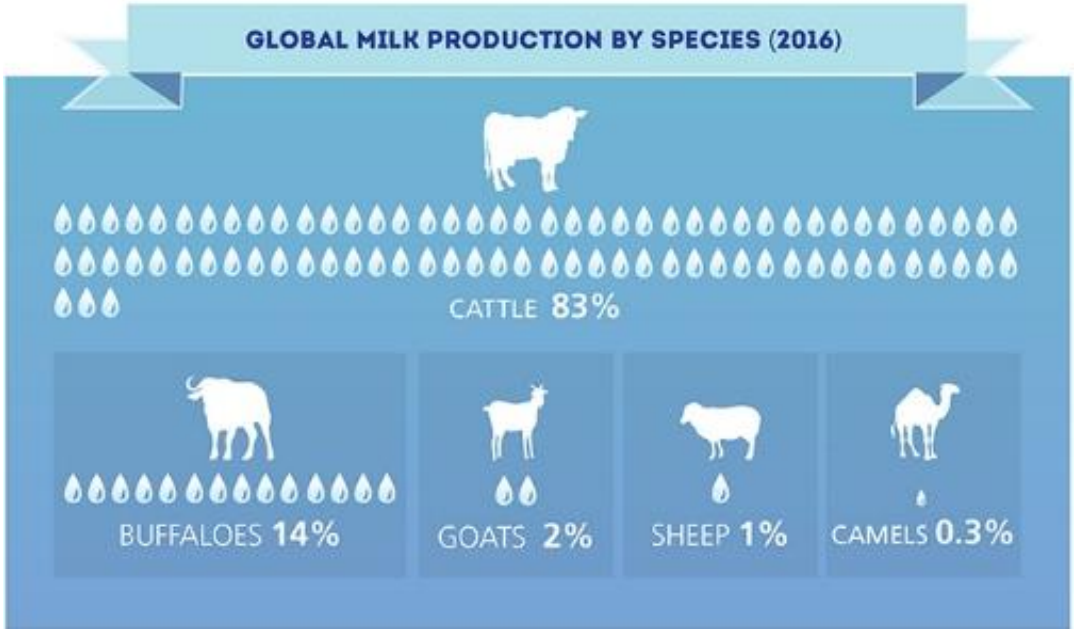

Figure 1.5 The Cow milk shares 83% of total Milk Produce of the world

(Source: http://www.fao.org/resources/infographics/infographics-details/en/c/273893/ ©: FAO**)**

Figure 1.5 emphasises on the reason why the research oriented towards quality monitoring of cow milk is important – 83% milk globally is in the form of cow milk, contributing to nine out of every ten glasses of milk consumed by people worldwide (Agriculture in the Classroom, 2018). This is primary reason why the scope of this project is targeted mainly towards the cow milk.

Quality, in general, could be more of a subjective term than objective and the idea of a good quality product can differ in each case depending upon the context it is discussed in, as well as what parameters are being considered to define and determine overall quality. Milk quality, in particular, could be defined in several ways too, e.g., for a common consumer the simplest form of a good quality milk product would be a product that appears normal, smells fresh and the one that tastes good.

However, the same might not be true if the idea of the said quality is scrutinised through some rigorous means of testing and monitoring the factors associated with the milk quality. This is also the reason why people with ill intention of making financial gains counterfeit milk with synthetic milk that tastes, smells and looks like milk but does not resemble the nutritional components that an ideal or normal milk product should have.

1.2 Importance of Milk Processing

Milk provides a very favourable environment, for growth of the microorganisms known as bacterial pathogens to further develop and breed, due to its high nutritional value. This phenomenon eventually causes spoilage of milk and practically leads to the short shelf life of milk and other dairy products made by processing of such milk. Apart of shortening the overall usable lifespan, bacteria adulteration of any milk products can also cause consumers to contract different food borne illnesses when these adulterated products are consumed.

For example one such story was reported in California (News Desk, 2015), related to the contamination found in raw whole milk, where the inspectors from California Department of Food and Agriculture confirmed the bacterial presence following the product testing as a routine inspection and sample collection from the facility. All the products of the said batch were ordered to be quarantined, followed by their removal and disposal from the store shelves, including those already sold and stored in customers' refrigerators.

Symptoms due to campylobacteriosis mainly include cramps in stomach, diarrhoea, or fever. These are observed generally after 2 to 5 days from being exposed to Campylobacter and last for around a week. Usually most persons with campylobacteriosis recover completely as this illness is mild, and some people with campylobacteriosis show no symptoms at all. Less likely, yet possible, symptoms could be joint pain and/or swelling.

Also, a rare disease known as Guillain-Barré syndrome, which brings weakness and paralysis may occur some weeks after the initial phase of symptoms. Another example is E. coli O157:H7 infection, normally associated with non-pasteurised milk, which develops symptoms of Haemolytic Uraemic Syndrome (HUS) that can lead to kidney failure as reported by News Desk, (2012).

In the year 2000, as many as 13,800 schoolchildren were affected in Japan after consuming skimmed milk that was contaminated. A power cut resulted in time–temperature tempering of the product. The company then inappropriately applied corrective action by an attempt to reheat the milk without realising that the S. aureus enterotoxins are heat stable once they are formed.

Motarjemi et al. (2014) list further similar incidents on foodborne disease outbreaks in their book chapter on milk and dairy products. These could be avoided to an extent by diligent handling, good storage conditions as well as milk processing. Milk processing enables its preservation for much longer periods and helps in reducing or avoiding food-borne illnesses resulting from the spoilage or lack of quality (FAO, 2015b). For these reasons, in the supply chain of dairy products, milk processing becomes the second very important stage at the industrial level after milk production at the dairy farms. It is important to note that the primary goal of milk processing is to help maintain the actual quality of raw milk and thereby to ensure its preservation.

Milk, which is improved in overall quality naturally, is preferred more over the milk that has artificially added nutritious values by means of processing. The high costs of production are not only because of the high value of raw milk alone but also due to its subsequent stages of milk processing that add to the overall retail value of the end product. Hence, Dairy industries, that procure milk from dairy farms to process the raw milk in order to produce pasteurised milk, cheese, butter, ice-cream etc., also play a very significant part in the overall economy of many developing as well as developed countries (Kenny, 2013).

1.3 Milk Supply Chain and Scope of the Research

In this section, the general structure of the milk supply chain is discussed while addressing the scope of this research within the supply chain. To understand where the significant challenges and main problems lie in the supply chain, it is necessary to study this hierarchy including their difficulties. It ranges from the procurement level, taking place at dairy farms, to the handling via the transport stage, and stretches to the storage at retail units followed by the end consumer level. Primary quality concerns arise at various stages of this entire hierarchy, including at the retailers that sell the milk products received after being processed and packaged from the dairy industries.

The tampering of quality, in the milk supply chain, generally takes place beyond the packaging and processing stage with some portion of real and fresh milk

being added with other malicious components such as detergents and urea, as explained in detail in section 1.4 of this chapter. The milk contamination could also result due to lack of diligent handling or done deliberately to prolong its shelf life and give false positives in basic routine tests. Such malpractice puts health of the consumers at severe risk especially when the presence of the harmful adulterants are not perceivable with organoleptic test as explained in Chapter 2.

It emphasizes on the fact that the proposed microwave sensing technology, although targeted towards the lower end of milk supply chain - beyond the milk processing and packaging stage, it could well be retrofitted to existing industrial setup due to its advantage of being low profile. Figure 1.6 illustrates the scope of proposed research in existing milk supply chain.

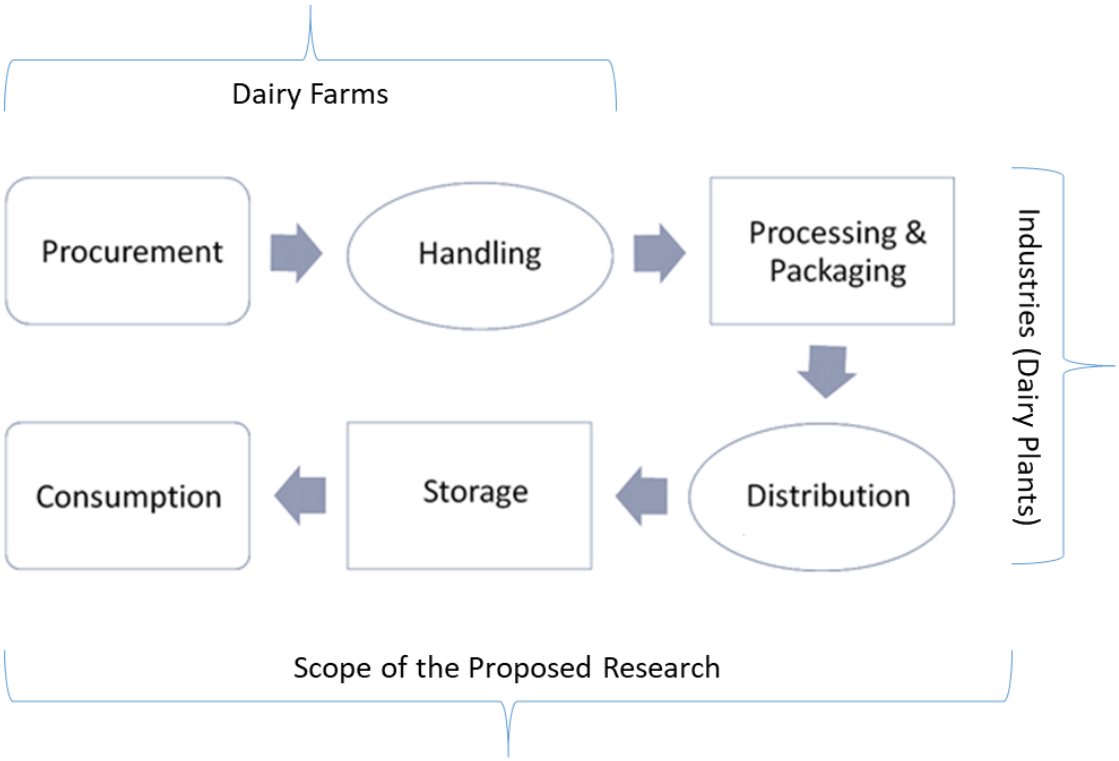

Figure 1.6 The scope of the proposed research in milk supply chain

As an example, testing is made at both levels, of procurement and packaging, by the industries for assuring the required quality, but at the end of each phase of transportation, it is likely that the checked quality of the milk product would have been altered. Specifically if the transport distance is long, the quantity of milk is large and the storage conditions are not maintained at required level.

The proposed sensor is targeted beyond the distribution stage of this chain, where food inspectors have to collect the samples to test and send it to laboratories owing to limitation of current standard practices. Furthermore, as the proposed technology is based on electromagnetic wave sensing, it could be easily retrofitted to existing robust industrial setup within the dairy industries.

Another big challenge, that raises concerns of milk quality and milk procurement, is fragmented milk production, where milk production takes place in an unorganised sector. This is prevalent over many countries like India – which is the largest producer as well as consumer of milk, having the largest livestock worldwide (NPCS Team, 2014). The research report by NPCS Team, (2014) also adds that milk supply chains face difficulties in transporting milk, especially in large quantity, under desired conditions over longer distances (>200km) while ensuring the quality of milk.

Figure 1.7 shows a representative image of such a fragmented sector where many small dairy farmers are sometimes dealing with customers directly, instead of with industries, and sell them the milk on regular basis on daily, monthly or yearly contracts. On the other hand, the European Union (EU) has one of the strictest food safety protocols in the world and hence even if there is any individual case of milk fraud, The Rapid Alert System, for Food and Feed Safety, feeds back the information when risks to public health are detected in the food chain (Handford, Campbell and Elliott, 2016).

Figure 1.7 A dairy farmer handling milk (representative image)

(Photo Credit: ILRI/Stevie Mann; https://www.flickr.com/photos/ilri/4028978890/**)**
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In addition, in the transport stage of the milk supply chain, it has been perceived that the milk samples can be adulterated with foreign materials which can cause severe health hazards, such as detergents which were found in 103 (8.4%) of the total observed samples.

Furthermore, because of poor hygiene and sanitation practised during milk handling and packaging, detergents (used for cleaning purpose) are not removed (indicating poor cleaning of milk containers) which eventually end up in milk (Handford, Campbell and Elliott, 2016). Hence, it is understood that the milk quality can deteriorate even post-production and post-processing stages due to certain poor conditions unfavourable for the quality maintenance of pasteurised and homogenised packaged milk.

This project is aimed at such stages beyond the industrial phase, once the milk product is processed and packaged for selling into market, where determination of milk quality and the monitoring process requires the technologies and systems to be very compact, reliable, non-invasive, real-time and non-destructive to save both time and resources.

1.4 Milk adulteration and its impact

The Food Safety and Standards Authority of India (FSSAI) defines adulteration in food as, ‘An act of intentionally debasing the quality of food offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredient’ (Food Safety and Standards Authority of India, 2014).

Adulteration can be mainly of the two following types -

- Intentional – done for commercial gains, e.g. adding water to pure milk or
- Accidental – occurring incidentally due to presence of bacterial organisms or foreign particles due to factors such as inappropriate handling and/or storage, both in pasteurised as well as non-pasteurised milk.

Around 68% of milk in India, which is the largest producer of milk globally with 16% share of world milk production (FAO, 2015c), doesn’t meet the recommended quality standards (Saxena, 2016).

As previously explained in section 1.2, on a regular basis from around the world, articles regarding the quality of milk, its contamination, spoilage and/or adulteration, with its possible adverse effect on consumers keep appearing in newspapers and other related media. The Hans India (2017) reported another similar story where local police in the city of Hyderabad arrested three businesspersons along with eight milk vendors for adulteration of milk with hazardous materials, which included some chemicals. In the operation, police seized a large quantity of Hydrogen Peroxide (H_2O_2), soda, salt (NaCl), milk powder and oil that were being used to adulterate the milk, evidently for making financial gains by artificially fabricating a product that could taste and look similar to pure quality milk.

Melamine, a synthetic chemical used in plastic coatings and laminates, was found to have been added to milk in China to boost its overall protein content. The Chinese Ministry of Health reported around 294,000 infants were caught sick after consuming the melamine contaminated infant formula, and more than 50,000 infants had been hospitalised with six deaths being confirmed (WHO, 2009). Melamine develops crystals in urine when its presence exceeds a threshold concentration. Several of the affected infants, in the above-mentioned incident in China, had developed kidney stones or calculi in the ureter or bladder and, hence, renal failure.

The intentionally added adulterants, for the purpose of commercial gains, include water, carbonates and bi-carbonates (such as $NaHCO_3$), starch, gelatine, and urea ($CO(NH_2)_2$). These have been reported to be a prevalent form of adulteration of milk in India, which is the largest producer as well as consumer of milk worldwide (Bhandare and Waskar, 2010).

A national survey conducted by FSSAI, in India, exposed water as the most widely used adulterant in milk, which greatly compromised the nutritional contents and hence the quality of milk samples. From all the non-confirming samples, 574 (46.8%) samples, belonged to the category of low solid non-fat (SNF) because of milk being diluted with water (Handford, Campbell and Elliott, 2016). The survey also proved that the second highest non-conformity was seen in skimmed milk powder, which was present in 548 (44.7%) samples, out of which glucose was

present in 477 samples, mainly added to enhance its SNF content.

Reports have also been made that the multinational firms, that are very strict in the quality maintenance of their products in developed countries, are allegedly adulterating milk and milk powder including infant meals as urea is a natural constituent in milk and its adulteration is easy (Dai *et al.*, 2010). From a total of 60 various milk samples collected from various public places as well as educational institutions in Faisalabad, Pakistan 63% and 87% were identified with urea adulteration, respectively (Afzal *et al.*, 2011).

In another work, a total of 365 children, in the age group between 1 and 22 years, from various urban and rural households, in Uttar Pradesh, India, were surveyed and samples were taken from each of these households (Bhatt, Singh and Bhatt, 2008). The researchers further collected 160 samples from local markets to analyse the presence of urea and detergent. The samples collected from urban regions showed higher levels of adulteration than the ones collected from rural areas, with children affected in all age groups suffering eyesight problems (57% in urban, 16% in rural) as well as diarrhoea, whereas headache was reported in the children within age group of 6 to 18 years. The age group of younger children within 1 to 5 years of age mainly relied on breastmilk and hence had reported the minimum of health related problems pertaining to affected milk consumption (Handford, Campbell and Elliott, 2016).

Urea is one commonly used adulterant because of the fact that it is a naturally found constituent, in milk as non-protein nitrogen (NPN), varying from 20 mg/100 ml up to 70mg/100ml, but any value beyond that 700ppm mark is indicative of urea which is externally spiked (FSSAI, 2012). Any higher amount of urea in milk than its natural component can cause a fatal health hazard.

1.5 Motivation, Aim and Objectives

1.5.1 Motivation and Need of Project

Recent years have seen a push towards an innovative approach in terms of milk spoilage prevention but this pursuit does not eliminate the need for future research into milk spoilage detection methods (Lu *et al.*, 2013).

There is a developing need for an accurate spoilage detection technique for processed and packaged milk as much as there is a growing demand to prevent the wastage of milk and the illnesses occurring due to contaminated milk consumption. The current state of the art and its detailed critical analysis is given in Chapter 2.

Based on the study of dairy production and agriculture programmes as well as school-based milk programmes, Muehlhoff, Bennett and McMahon (2013) report that the problems related to suitable levels of fat, added sugar and flavouring in milk are still required to be addressed. There is no effective yet easy-to-use and affordable method currently existing to achieve these goals (Lu *et al.*, 2013). In the late 1990s and early 2000s, the evolvement in high-speed instrumental testing techniques to measure dairy and packaged milk composition provided the required platform for enhancement in the overall effectiveness and accuracy of traditional chemical analysis methods (Barbano and Lynch, 2006).

The motivation of this research work is to introduce a novel, rapid milk quality monitoring technique, using a non-invasive, non-destructive, real-time approach incorporating microwave sensors, which is less complex, accurate and adding further to the current technologies in a time and resource saving manner. This technique aims to cover three aspects of quality testing, namely spoilage detection, classification of milk in terms of nutritional constituents present, and adulteration check, within a single system. There is no technique, currently available, incorporating all of these three elements of quality control under one system. Based on the literature reviewed, it was apparent that there is little in the way of rapid measurement for the spoilage, constituent and contamination (adulteration) of milk by a single technique.

Those systems, which do exist in the research or commercial domains, are largely laboratory based or require significant cost for installation. Therefore, the purpose of this work is to investigate the use of EM wave sensors, operating at Radio and microwave frequencies, to provide real-time solutions for the milk quality testing. Hence, developing novel industrially focussed sensor system, which is compact, less costly and readily retrofitted to current processes for milk quality testing in real-time environment, is the main drive of this work.

1.5.2 Aim of the Research

The aim of this research is to develop a novel, microwave sensor system for monitoring overall quality of pasteurised and homogenised cow milk in terms of spoilage determination, classification of milk types based on composition, and identifying adulteration. The aim is targeted beyond the dairy plant stage after milk is processed and packed for the retail selling. Figure 1.8 breaks down the main aim of this research work broadly.

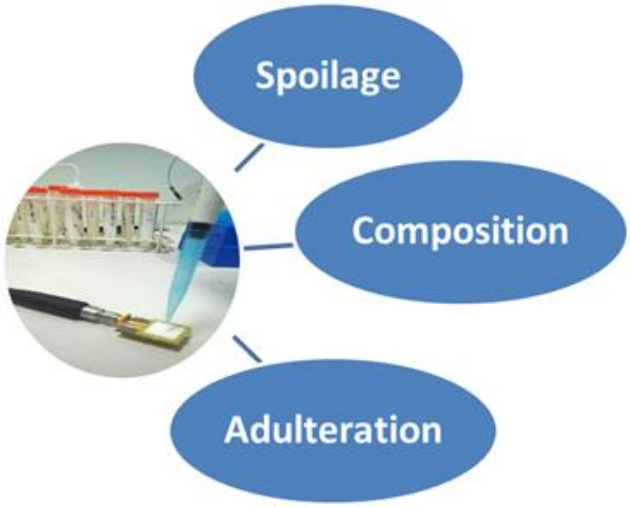


Figure 1.8 Aim of the project to determine the Quality of Processed and Packaged Cow Milk

1.5.3 Objectives of the Project

To achieve the aim of this project for determining the overall quality of packaged cow milk, using EM wave based sensing technique, following objectives were established:

- Investigate the problems faced by dairy industry for quality monitoring of processed and packaged milk and the limitations of current state of the art in industry as well recent advancements in the research domain.

- Investigate the EM wave theory, its advantages and study of the currently existing microwave sensors to establish the scope of this research work.

- Determine the dielectric property of the freshly bought milk types (skimmed, semi-skimmed, whole) for a week's period to understand the dielectric property changes with respect to milk types (i.e. contents) as well as the occurrence of spoilage over time.

- Design and simulate theoretical model of the sensor based on the dielectric property data using ANSYS High Frequency Structure Simulator (HFSS).
- Design and fabricate a lightweight and portable microwave sensor, ideally a planar sensor of few centimetres size; carry out the experimental test measurements for spoilage determination, milk type classification, and adulteration with the help of common and easily undetectable adulterants.
- Analyse the recorded EM spectral data to determine the milk spoilage, milk type classification, and adulteration using novel microwave sensor.
- Make comparative review of the microwave sensing technology with regards to the existing technologies and give recommendations of choice of microwave sensor design for processed and packaged milk quality control.

1.6 Statement of Novelty

This thesis describes the development of low-cost, low profile, non-invasive, non-destructive, time and resource efficient microwave fluidic sensor system for monitoring of milk quality by measurements of spoilage, composition and adulteration, which is currently not available for the packaged milk analysis beyond dairy processing and packaging stage. This research work demonstrates rapid measurement technique for milk quality control incorporating, spoilage detection, content based milk type classification, and determining adulteration through a single sensor-system. This will help to eliminate the time- and resource-consuming stages in several currently used methodologies, such as requirements of preparing and adding reagents.

1.7 Organisation of the Thesis

This PhD thesis consists of eight chapters as described below:

Chapter 1 provides a brief introduction about the whole research project by discussing the background and its primary aim, objectives and motivation for the need to implement this research work along with its novelty.

Chapter 2 describes the milk quality control with the current state of the art in the dairy industry as well as recent advancements in research domain, with their critical review, highlighting the advantages and disadvantages of each of the techniques studied.

Chapter 3 discusses the advantages and principle of using microwave sensing technology over the other existing approaches as explained in Chapter 2. In addition, existing microwave sensor designs and its applications are briefly discussed followed by highlighting the design considerations for this research work.

Chapter 4 discusses the dielectric property of milk. The methodology adopted for dielectric property test, of three milk product types, namely skimmed milk, semi-skimmed milk and whole milk, is explained along with results and discussion. Microwave sensors used in this research work are simulated, based on the spectral signatures data recorded from dielectric property tests in this chapter, followed by, design of a low profile, non-destructive, non-invasive planar resonator type fluidic sensor for milk quality monitoring technique.

Chapter 5 shows the use of a variety of EM wave sensors to detect spoilage and periodic deterioration of milk over time. The methodology adopted and sample preparation are explained followed by the discussion of test results achieved for the measurements of spoilage detection. The analysed data are shown in graphs for further interpretation.

Chapter 6 gives the methodology based on microwave spectroscopy to distinguish among types of milk samples based on their content. The primary focus was on fat content of milk, based on which packaged milk types are commercially classified. Results for varying protein content are also evaluated and discussed.

Chapter 7 studies the adulteration of milk with urea, and detergents in deionised water as cleaning agents, as forms of adulteration. The results show the concentrations of adulterants as they grow in volume. Liquid Gold and Acid Descaler were used as alkali and acidic detergents, respectively, as used by milk industries to wash large containers carrying milk before refilling cycles.

Chapter 8 presents the critical review of the entire research project in comparison of the existing technologies, and shows where the proposed technology stands with regards to the current state of the art. The recommendations related to suitable choice of the type of microwave sensor within EM wave sensing technology, for processed and packaged milk quality monitoring, are also made in this chapter.

Chapter 9, based on the results achieved from various microwave sensors, including the fluidic planar resonator sensor for milk quality tests, summarises the research work with regards to the research objectives laid out in Chapter 1 and the concludes the project. Further expansion work is also suggested to improvise the prototype design.

1.8 Summary

This research work seeks to provide a proof-of-concept solution, which could serve effectively and be employed beyond the dairy industry's robust setups at the consumer end of the milk supply chain hierarchy. This means that the primary focus of this work is aimed towards the user end of the production chain and not at the milk procurement level, where dairies already have the robust quality assessment techniques existing within the industries as explained in Chapter 2.

This project is targeted towards the rapid measurement technique quality determination of packaged skimmed, semi-skimmed and whole milk, which is non-invasive, non-destructive and can be applied at food quality and testing laboratories. The aim of the research is to further aid the product quality inspection such as the tests done by food inspectors, or by medium and large scale retailers, who receive the milk from dairies for selling in packaging, to help allow the quality assurance and also to ensure that the customers are getting what they are paying for.

The food inspectors can implement random checks for the packaged products from a given batch, as the proposed methodology addresses all three aspects of milk quality control in terms of spoilage detection, milk type classification and

adulteration check. This can eventually eliminate the need to check every single package of the same batch and hence not every end user need to have this application in hand to assess the quality of milk as it could be broadly applied at the link just before the very end consumer.

CHAPTER 2

MILK QUALITY CONTROL: CURRENT STATE OF THE ART

2.1 Background

This chapter discusses milk processing and standard quality control techniques generally performed on raw milk to preserve its natural nutritional value to acceptable standards, which prolong the shelf life ensuring that the processed milk is safe to consume and free from elements like bacterial pathogens, contamination and spoilage. As discussed in the previous chapter the risk of foreign particles or microorganisms adulterating the milk is more when milk is raw and not processed to filter out any inherent bacterial pathogens that come along from the farm during the procurement stage. This chapter further discusses and critically analyses the recent advancements in milk quality monitoring techniques while highlighting the gaps and limitations within existing practice.

Milk quality control is the application of approved testing techniques, ensuring standards related to the milk and milk products are regulated to expected levels. This also takes into account the composition, purity and the level of different microorganisms present within milk. Broadly, it covers testing milk and milk products for quality as well as monitoring that - milk products, milk processors and the marketing agencies involved adhere to the accepted codes of practices (Ministry of Agriculture - Republic of Kenya, 2011).

Each country ideally has a general set of guidelines outlined in a specific legal document normally governed by a regulatory body or respective food and quality control agencies of the nation. In the EU, food hygiene legislation, with effect from 1 January 2006, sets out clear duties of food businesses in safe production while being consistent at it. That covers the entire food chain from the farm to the fork (Food Standards Agency, 2016). For England and Wales the Food Standards Agency (FSA), defines “A Practical Guide for Milk Producers” under The Food Safety and Hygiene (England) Regulations, 2013 and The Food Hygiene (Wales) Regulations, 2006. Similarly, in India the regulatory body Food Safety and Standards Authority of India (FSSAI) sets out instructions and standard protocols.

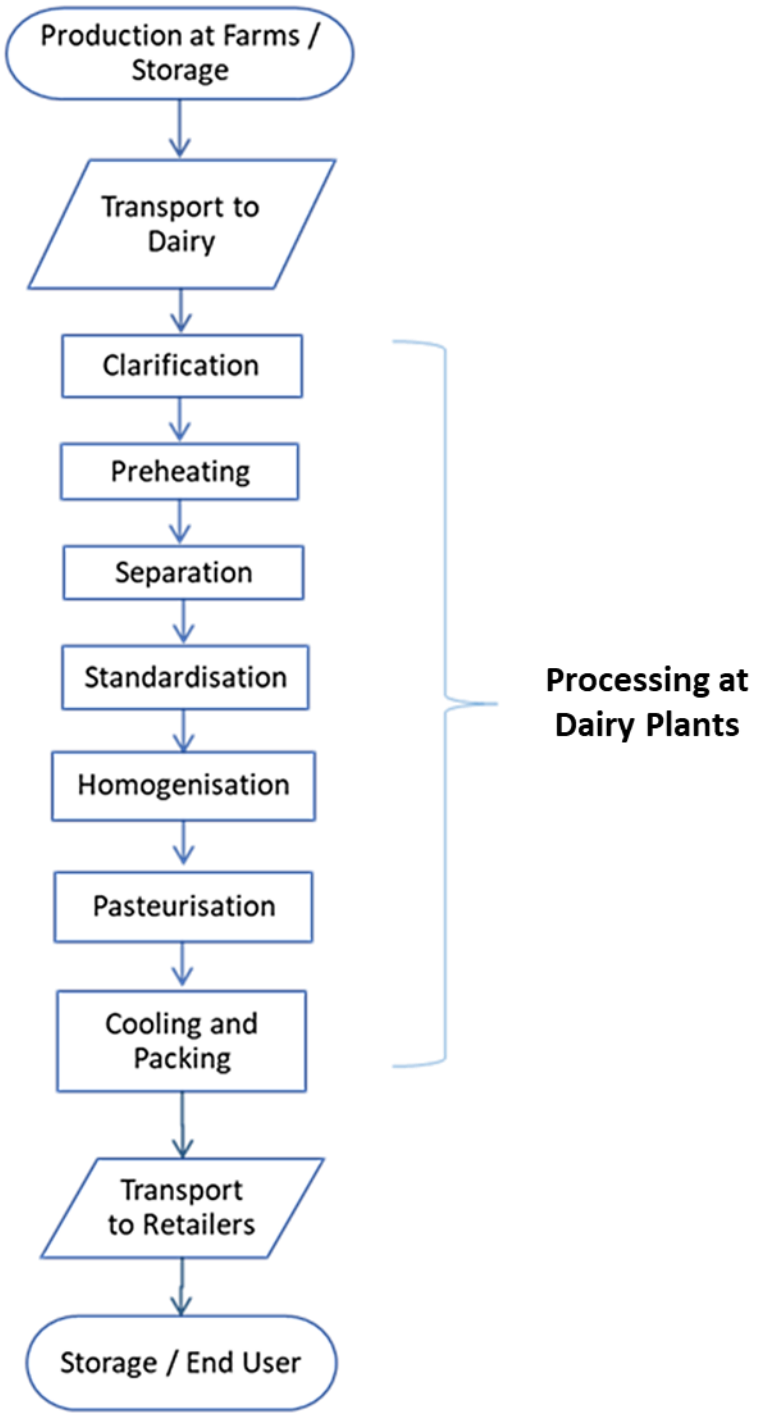


Figure 2.1 Basic flow-chart of Milk Production and Processing

In the commercial market, the milk producers would expect a fair price according to the quality of milk that they produce, whereas the milk processors pay the producers, having been assured, that the milk received for processing has normal composition, as well as being suitable for processing into different dairy products. The consumer eventually expects to pay a fair price for milk and various other milk products that could range between acceptable and excellent quality.

Figure 2.1 gives a general overview of the milk production chain in the form of a process flow-chart. The significance of studying this procedural hierarchy is to emphasise on the fact that there already exists a robust mechanism to deal with quality issues related to the raw milk at industrial levels. Hence, the focus of this research is targeted specifically beyond the milk processing and packaging stage.

Milk contamination can take place in different ways, including by dairy cattle directly shedding the organism in their milk. Hence, milk refrigeration right after milking is important for controlling the growth of these organisms. However, considering the fact that, microorganisms such as L. monocytogenes can survive adverse environmental conditions, even at low temperatures, the collected milk from the dairy farms should be processed as soon as possible (Motarjemi *et al.*, 2014).

The procured milk undergoes a number of operations in the dairy plant starting from storage and followed by processing stages such as clarification, preheating, separation, standardisation of fat content, homogenisation, pasteurisation, cooling and finally packaging. The raw milk is stored in silos for a limited time below 7°C as per local regulatory requirements as it is essential for limiting the growth of organisms. Pasteurisation is very important for the safety of raw milk, which is received from farms, to eliminate foodborne pathogens. Vegetative cells of foodborne pathogens are sensitive to heat and, therefore, the most heat-resistant pathogens like C. burnetii, M. tuberculosis and L. monocytogenes, are killed during the pasteurisation stage. Other pathogens namely Brucella, Campylobacter, E. coli, Salmonella, S. aureus and Yersinia are also killed during this stage. Spoilage bacteria and undesirable enzymes (lipases as well as protease) are also reduced during the pasteurisation process, ensuring safety of the milk product as well as prolonging the shelf life with minimum changes to its flavour and nutritional quality (Motarjemi *et al.*, 2014).

Low Temperature-Long Time (LTLT) method is used in batch pasteurisation of milk while it is stirred continuously at 63°C for a minimum period of 30 minutes, whereas the High Temperature-Short Time (HTST) is more energy efficient and applied for a minimum of 15 seconds at 71.7°C in heat exchangers. The limitation of pasteurisation is that it does not eliminate bacteria spores, and many other spoilage bacteria are actually resistant to the temperatures used in pasteurisation.

To destroy these endospores, higher heat treatment such as Ultra-High Temperature (UHT) must be applied at 135°C for 1 second (Motarjemi *et al.*, 2014). The UHT processed milk can be stored unrefrigerated, and has a long shelf life, provided it is unopened and packed with aseptic packaging. The pasteurised milk on the other hand has to be refrigerated as soon as possible after being processed to prevent development and growth of S. aureus and thereby production of heat stable enterotoxins.

2.2 Milk Quality Control Techniques in Industries

In the first section, the techniques, which are widely used at present in dairy industries, are discussed in general. This allows the understanding of the ways in which the practical application of milk quality checks is carried out currently in industrial domain, at large. The simplest form of milk quality sensing is the manual sensory method that simply involves sensing the change in smell, colour or texture of the milk product. There are various milk quality testing techniques being applied and experimented within laboratories and industrial levels.

The tests focused on determination of adulteration in milk are predominant in the industries; besides these, certain general tests are carried out to find overall milk quality, which include tests like the phosphatase test done on pasteurised milk and the acidity development test used for Ultra High Temperature (U.H.T.) processed milk (Ministry of Agriculture - Republic of Kenya, 2011). Interest is also increasing in techniques which are aimed specifically at checking the contamination within milk, such as Detergent Residue Testing (Dunsmore, 1983).

In the work done by Deeth *et al.* (2002), spoilage patterns for skimmed and whole milk are studied and distinguished, using a manual sensory method first and then, using Gram strain and oxidase test, preliminary identification of spoilage bacteria was made, followed by identification of the isolates through an Analytical Profile Index (API) supplied by Bio Merieux S.A., France. The preliminary identification was carried out through an oxidase test and rating was done with averaging of visual analysis and the smell of milk samples performed by two different qualified milk graders on a scale from 0 to 9.

The samples were then also sent to a laboratory for total aerobic count of bacteria and further analysis. The milk that scored less than or equal to 1 and hence termed as grossly spoiled, was later isolated from corresponding agar plates for bacteria isolation. The other commonly used approaches in milk industry are briefed as follows (Ministry of Agriculture - Republic of Kenya, 2011):

2.2.1 The Organoleptic Test

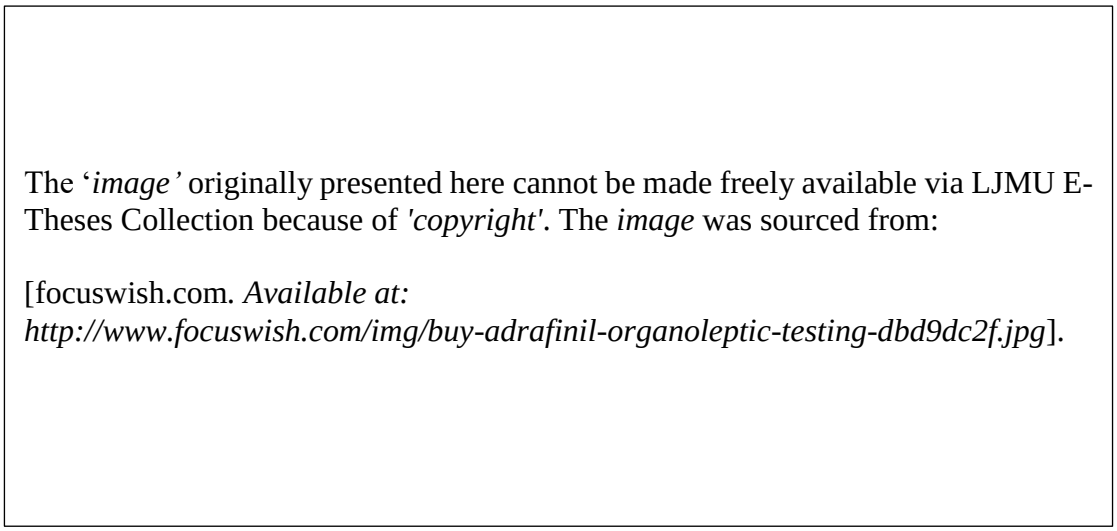


Figure 2.2 Organoleptic Analysis (the visual inspection of a compound)

As shown Figure 2.2 in this test, no equipment is required, but the milk grader must have a good sense of sight, smell and taste. Janzen, Bishop and Bodine (1982) applied such tests on milk samples with the help of two experienced judges from the American Dairy Science Association (ADSA), who graded milk samples with flavour score under 6.0, out of a scale of total 10, as 'unacceptable'.

Hence, milk samples, which cannot be sufficiently judged through the organoleptic test, must be subjected to other more sensitive and objective tests. Nicolaou-Markide (2011) employs a similar organoleptic test besides pH and Total Viable Count (TVC) to analyse the results achieved. These tests are basic approaches and cannot detect odourless and tasteless adulterants such as urea.

2.2.2 Acidity Test

Overall acidity content of milk is increased as it spoils. Hence, acidity level is quantified to enable milk quality measurement. For dairy products, acidity content can be expressed in two ways - titratable acidity (as shown in Figure 2.3), which shows total acidity (not acid strength) and hydrogen ion concentration (pH), which indicates acid strength (Lu *et al.*, 2013). Bacteria developing in raw milk normally produce more or less lactic acid. From this, the lactic acid percentage can be calculated. The natural acidity of milk is 0.16 - 0.18%. Therefore, with the help of this test, it is revealed that any figures higher than this would be an indication of the developed acidity due to action of bacteria present.

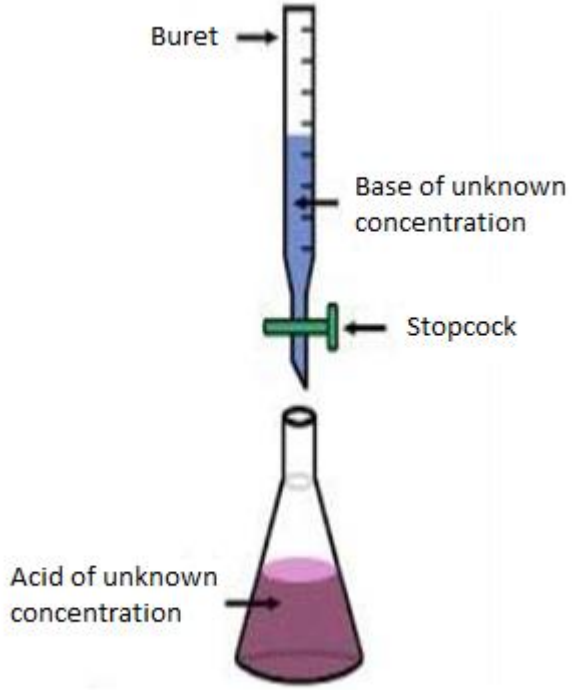


Figure 2.3 Milk titratable Acidity Testing apparatus

(Source: http://img.sparknotes.com/figures/3/3a5994498f24d59f5d5d762b40844a2a/titsetup.gif, **Credit**: SparkNotes.com**)**

There are different ways of measuring milk pH value. While a pH meter that gives a definite value, pH indicator paper strips are used for general estimate of milk acidity by dipping a strip into the milk sample and then matching the colour obtained by it to those in the colour chart. Another form of pH testing includes pH indicator kits where universal pH indicator solution is added to the milk sample and its colour

change is matched with a colour palette chart ranging from acidic to alkaline. Among these existing current techniques, pH Indicators mainly focus on the pH level detection, and may or may not always represent the perceivable spoilage of the sample. Based on pH value detected, they aim to determine the deterioration which leads to inaccurate results because pH levels easily fluctuate and the pH of unspoiled milk is around 6.7, which is also the pH value at which many forms of bacterial micro-organisms develop (Klaenhammer, 1988). At lower pH values between 4.0 - 5.0, lactic acid bacteria can grow and produce lactic acid, which is the phenomenon used for fermentation of milk for making various other dairy products.

2.2.3 Clot on Boiling (C.O.B) Test

If milk is stored at room temperature, there is increased acidity in that milk which is referred to as developed acidity. Any such increase of milk acidity by more than 0.2 percent, will introduce coagulation in milk due to heat treatment, taking place due to calcium dissociating from caseinate salt (My Agriculture Information Bank, 2015). Therefore, it becomes necessary to know the heat stability of incoming raw milk for its suitability for further processing. This is one of the old tests for too acidic milk (pH<5.8) or abnormal milk (e.g. mastitis milk).

Figure 2.4 Milk sample clotted at the end of Boiling Test
(Indication of acidic milk)

(Source: https://allaboutbiologyworld.blogspot.co.uk/2017/07/cob-clot-on-boiling-test.html,
Photo Credit: Ahmed Ashraf**)**

Figure 2.4 shows a positive result with the presence of clots at the end of the boiling test upon the milk sample under consideration, indicating that the milk is acidic. This means that the milk sample has failed in the test, the milk must contain many acid or rennet producing microorganisms or the milk has an abnormally high amount of proteins like colostral milk. Such milk cannot stand the heat treatment in milk processing and must, therefore, be rejected.

2.2.4 The Alcohol Test

It is based on the instability of the proteins when the levels of acid and/or rennet are increased and acted upon by the alcohol. It is used as a rapid determination of the elevated acidity of milk, when the milk sample seems sour from the organoleptic test. If the test results show too high acidity in milk then the sample is sent to the lab for further titratable acidity testing (Siirtola, 2000).

This test relies on the phenomenon that the proteins, in sour milk due to lactic acid formation by bacteria, become susceptible to alcohol precipitation. If the mixing of equal quantities of milk and 68% alcohol in a test tube results in coagulation of proteins, then it indicates that milk has become sour and, hence, is not fit for any procedures that apply pasteurisation on it. This is because proteins, in milk with elevated acidity, have also loosed the heat stability against the temperatures used for pasteurisation. For this reason, it is recommended that the alcohol test should be applied to each incoming milk container if the milk is to be treated for pasteurisation.

The Alcohol-Alizarin test is procedurally the same as the alcohol test but this test is more informative. Alizarin is a colour indicator which changes colour as per the acidity level (Ministry of Agriculture - Republic of Kenya, 2011).

2.2.5 Resazurin Test

It is the most widely used test for hygiene and the storing quality of raw milk. Resazurin is a type of dye indicator. Under specified conditions Resazurin is dissolved in distilled, boiled water. This solution is later used to examine the microbial activity in a given milk sample. With the growth of bacteria in milk, the redox potential of the milk also changes with time and the general trend for potential to change is in a negative direction.

A rapid change is seen after the dissolved oxygen in milk has been consumed by aerobic type bacteria, and can be determined by a change in colour of certain dyes added in the milk sample.

These dyes are oxidants of redox systems, forming the basis of methylene blue and Resazurin reduction tests for quality of milk, in terms of bacterial presence (Bhandari and Singh, 2002). As the time that passes before these dyes are reduced to a colourless reductant form, the colour reduction is approximately proportional to the number of bacteria present. Hence, this reduction time becomes an index for the degree of bacterial contamination. Figure 2.5 shows the patent experimental setup image as filed in 1946 (Golding, 1952).

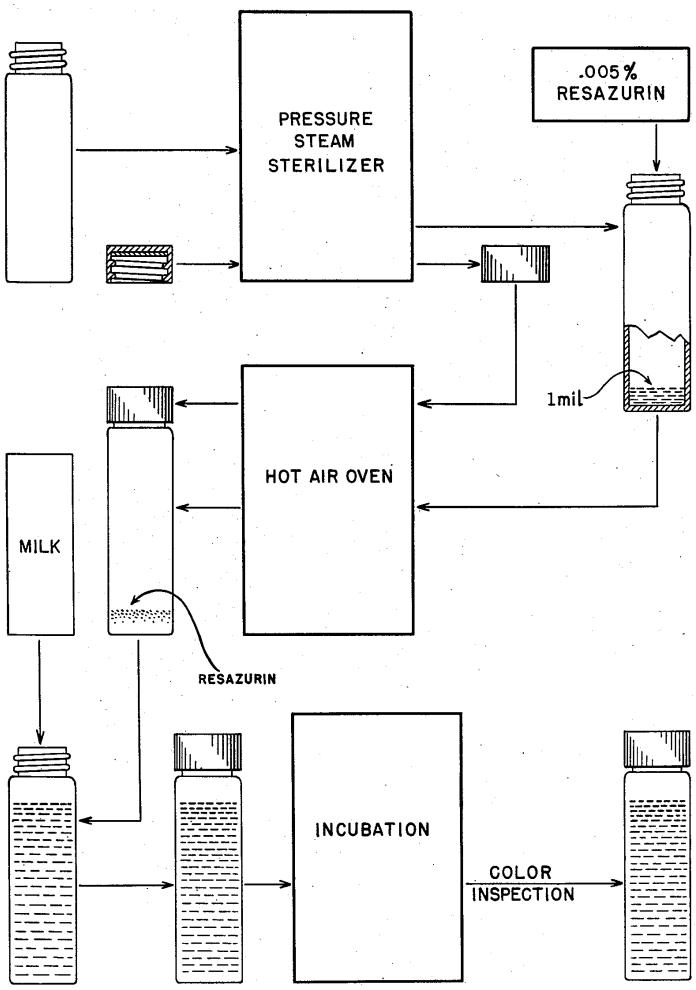


Figure 2.5 Resazurin Test setup for Milk as filed in patent by Norman Golding

(Source: https://patentimages.storage.googleapis.com/pages/US2609275-0.png,

Credit: United States Patent and Trademark Office, https://www.uspto.gov/**)**

2.2.6 Fat and Protein Analysis Methods

The Gerber Butterfat tests are done in order to make accurate adjustments of the butterfat percentage in standardised milk and milk products. Lipid/Fat content is determined by the Gerber method (or modified Gerber method; Troy Fucoma) in Europe where dairy product is added into a butyrometer with Sulphuric acid and Amyl alcohol, both behaving as dissolvent agents to the non-fat milk solids (Kleyn *et al.*, 2001). Another improved approach, the Mojonnier method, is accurate but very costly (Moore and Morse, 1926). In the US, a very similar method called Babcock is used. Gravimetric estimations like the Rose-Gottlieb method achieves higher accuracy as milkfat is extracted using solvents and weighed, which makes them independent of butterfat composition (Houston, 1955).

Protein or fat counts is another approach some researchers have used to account for the spoilage in milk by measuring the protein/fat counts as explained by Yagoub, Bellow and El Zubeir (2008). Pseudomonas aeruginosa (P. aeruginosa) was correlated to proteolytic activities in all food systems by many other studies as well. It not only produces a significant amount of proteinases and lipases but also plays a vital role in producing by-products by taking part in lipid and protein breakdown along with temperature effects and storage time conditions (Kohlmann *et al.*, 1991). Hence, a good amount of technical ability and knowledge is required, which makes this method less favourable amongst end users.

Nicolaou-Markide (2011) specifically focuses on the adulteration of milk and especially on the various types of data analysis techniques done on the captured data, whereas, Reinemann and Helgren (2004) describe the lack of intelligent approaches in milk sensing technologies currently being used. As after the determination of good or poor quality of milk, most of the systems do not offer the automated diversion of poor quality of milk to maintain the high standards of their products at the manufacturers' end. The industrial approach to determine Protein levels known as the Dumas method is an enhanced version of the standard Kjeldahl method and hence more preferred (Chiacchierini *et al.*, 2003). Both the methods need to convert measured nitrogen concentration to a protein concentration, as they do not directly measure the protein, which leads to inaccuracies.

Other fat analysis techniques include Gas Chromatography (GC) techniques, which are now enhanced from conventional techniques to high speed for industrial application due to being relatively bulky as well as highly expensive. Povolo and Contarini (2009) employed such fast GC for milk fat purity analysis of cows' milk. Whereas Dai *et al.* (2010) made analysis of urea in milk and milk powder by isotope dilution gas chromatography–mass spectrometry.

2.2.7 The Lactometer Test

The density of milk changes from its normal value to an abnormal value when water or other materials are added to it. The lactometer test is designed to detect the change in density of such adulterated milk. This is one of the basic types of test to detect simpler adulteration affecting the density of milk. Singuluri and Sukumaran (2014) report that the sodium chloride is used particularly to manipulate the lactometer readings, to enable false positive measurements, masking the added water in milk. Figure 2.6 shows a hydrometer as used in lactometer test to find milk density.

Figure 2.6 Hydrometer used in lactometer test to measure density of milk sample

2.2.8 Inhibitor Test

Milk collected from producers may contain drugs and/or pesticides residues. These, when present in significant amounts in milk, may hinder the growth of lactic acid bacteria used in the making of fermented milk products such as cheese

and yoghurt, in addition to being a health hazard. This method tests the suspected milk sample to determine and distinguish between any changes. Kohlmann et al. (1991) determined the effect of known protease inhibitors on protease activity.

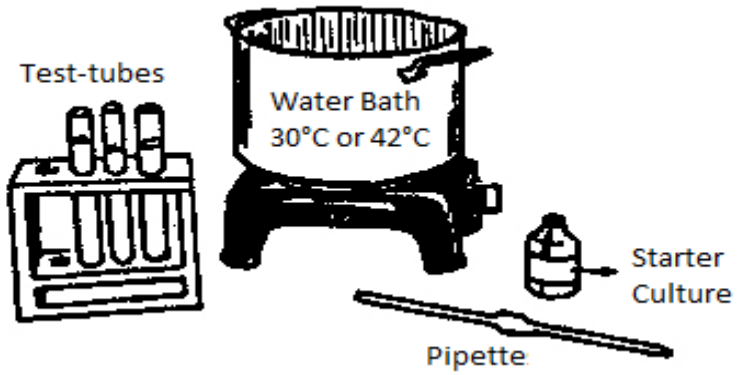


Figure 2.7 Apparatus required for Milk Inhibitor Test

(Source: http://www.fao.org/ag/againfo/resources/documents/MPGuide/Image30.jpg, ©: FAO**)**

Figure 2.7 shows apparatus involved in the inhibitor test for milk where the milk sample under test is subjected to a fermentation test using starter culture and its acidity is checked after 3 hours. The titratable acidity value, obtained, is then compared with that of a similarly treated sample which is free from any inhibitory substances, i.e., normal milk sample (Ministry of Agriculture - Republic of Kenya, 2011).

2.2.9 Freezing Point Determination

This test is done by simply observing the freezing point of a given milk sample. The freezing point of milk is known to be the most consistent of all measurable milk properties. A small adulteration of milk with water will cause a detectable elevation of the freezing point of milk from its normal value, which is -0.54 ºC. The adulterated milk by addition of water may have also been standardised by adding skimmed milk or partially skimmed milk. Even in this case this test can detect the variations because of the fact that when solutes are dissolved in an aqueous solvent, the freezing point of the solvent is lowered. Such lowering is normally directly proportional to the concentration of solutes added in the solvent (Bhandari and Singh, 2002).

2.3 Milk Spoilage Detection Techniques in Research Domain

In this section, other research works and techniques related to milk quality testing, in terms of milk spoilage detection, are discussed in brief and critically analysed at the end.

2.3.1 Lipid based Disposable Sensors

Another approach has been developed where lipid based, disposable, screen-printed sensors are employed along with the support of Principal Component Analysis (PCA), which is a pattern recognition technique, using SPSS (Statistical Programme for Social Scientist). Figure 2.8 shows a cross-sectional view of the screen-printed lipid membrane sensor as used by Mee Sim et al. (2003).

In this design, the working electrode as well as the reference electrode were integrated onto a single strip, which comprises of eight circular graphite working-electrodes and an Ag/AgCl reference-electrode. The disposable strip was printed on a polyester planar substrate and nine conducting paths were printed with silver ink.

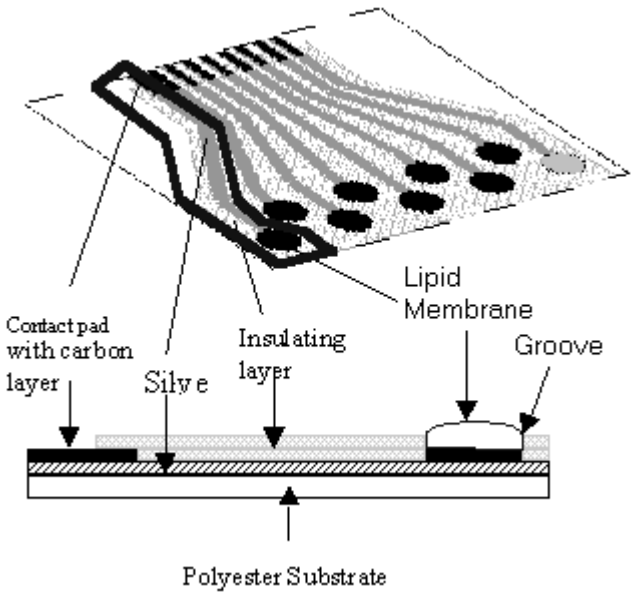


Figure 2.8 Disposable Taste Sensor System (Schematic)

(Source: Mee Sim et al., 2003**)**

The disposable sensors were rinsed with distilled water before being immersed

into the pasteurised and UHT milk samples bought over the counter. From screen-printing to acquiring results followed by processing and distinguishing among the results, this process requires highly technical supervision, making it complicated for the common market use. In this technique also, the primary focus was on the detection of spoilage and not on the type of milk. Due to the excessive technical knowledge required regarding the lipid materials to be used while designing the disposable sensor and then setting up the taste sensor system, this approach is not so favourable for real-time and rapid testing systems.

2.3.2 Gas Sensor Arrays

Gas Sensor Arrays, specifically aim to detect the strains due to the strong volatile compounds; moreover, they require a very large and costly setup. These arrays normally involve solid state, i.e. Metal Oxide Semiconductor (MOS) and MOS Field Effect Transistor (MOSFET) or IR based CO_2 gas-sensor arrays, as used by Haugen et al. (2006). The approach is to detect volatile bacterial metabolites by predicting the strain cultures that a certain compound produces.

The highest CO_2 readings were recorded in the pure S. marcescens N9 culture and in the mixed culture, whereas the pure Pseudomonas culture contributed a relatively lower production of volatiles including also CO_2. This operational behaviour of gas-sensors inflicts a limitation on overall testing system, as for every individual volatile compound produced by different bacteria – it is required to establish a dedicated gas-sensor to detect resulting strains. Moreover, in the case of bacteria that generate lesser volatile compounds, the sensitivity of the sensor has to be potentially high in order to be able to sense the low number of strains.

2.3.3 IR Spectroscopy

An Infra-Red (IR) spectrometer produces IR light over a range of wavelengths and monitors the vibration of molecules. Figure 2.9 illustrates the schematic diagram of an FT-IR spectrometer (Subramanian, Prabhakar and Rodriguez-Saona, 2011). The general setup comprises of a source that produces IR light, an interferometer that generates a range of wave numbers, and a detector that records the signal.

The interferometer is the key component of a spectrometer, which comprises of a fixed mirror, moving mirror as well as a beam-splitter. The beam-splitter splits an incoming IR beam followed by recombining it to produce varying IR wavelengths. A combination of mirrors is used to deflect the beam, whereas the laser acts as a time reference for data collection. The detector records the signal as an interferogram, which is later Fourier-transformed resulting in a single-beam spectrum. Fourier transform converts recorded data to absorbance and transmittance from a frequency and wavelength form.

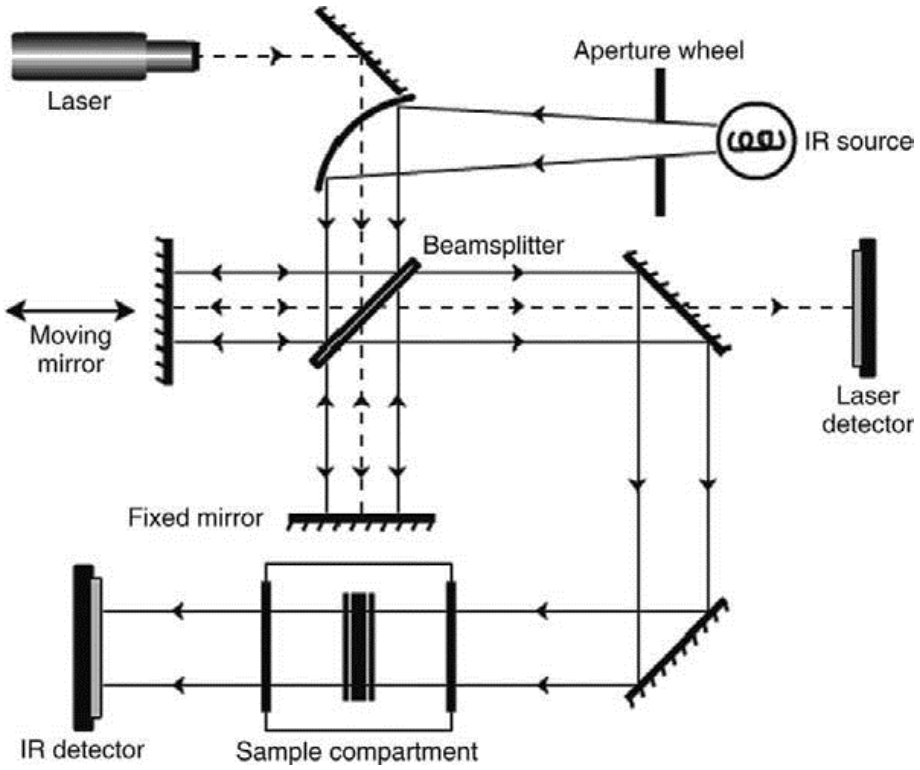


Figure 2.9 Optical layout of a typical Fourier Transform Infrared Spectrometer

(Source: Subramanian, Prabhakar and Rodriguez-Saona, 2011**)**

IR Spectroscopy, in general, is less used in the industries than the other methods and also requires a costly laboratory setup. The biochemical changes occurring in milk due to bacterial growth could be detected by visible and Short Wavelength Near Infra-Red (SW-NIR) diffuse spectroscopy (600nm-1,100nm) to distinguish between good-quality and spoiled milk samples without requiring to enumerate bacteria by monitoring quality loss in pasteurised skimmed milk (Al-Qadiri *et al.*, 2008).

As high as about 90% accuracy was achieved using multivariate data analytical techniques such as Principal Component Analysis (PCA), Soft Independent Modelling of Class Analogy (SIMCA), and Partial Least Squares (PLS) under segregated storage treatments (6°C-37°C storage for 0h-30h). The main drawback with this method is its high installation cost of experimental setups. This procedure again focuses on spoiled and good quality milk. The pH count and bacterial proliferation were used to compare with various spectral features. Again, a spectrometer was used to acquire the NIR spectra using a fibre optic set-up, making the design costly and complicated, as the acquired data needs to be mathematically processed to give any usable correlation.

2.3.4 Amperometric Method

This method monitors the growth of coliforms in milk by measuring the current change in an amperometric sensor. The sensor designed by Lee et al. (2009) comprised of a circuit with a potentiostat and two electrodes which were immersed in milk samples containing Methylene Blue with various concentrations of bacterial inoculums.

The microbial metabolism leads to the reduction of Methylene Blue, gradually, resulting in a change of current. The Detection Time (DT) required for sensing this detectable change of current gives the measure of present microorganisms available at any given time. This method is simpler compared to conventional bacteria plating methods but it requires the supervision of skilled worker and hence it is time consuming and not preferred.

2.3.5 Magneto-elastic Sensors

Wireless Detection, in terms of a remote-query technique to detect spoilage of milk is again an emerging field (Lu *et al.*, 2013). Remote-query sensor, as used by Huang et al. (2008) to measure the bacterial count of Staphylococcus aureus ssp. Anaerobius (S. aureus) in milk, is ideally a stand-alone, magneto-elastic sensing layer comprising of ribbon-like thick-film linked with some chemical or bio-chemical. Here, S. aureus are the bacteria that reside in milk and multiply as milk spoils; infection with S. aureus can result in such human diseases as toxic shock syndrome, endocarditis, and septicaemia (Guntupalli *et al.*, 2007).

The sensor was placed inside of a 2 ml cuvette that contained milk sample followed by within a solenoid coil used for signal telemetry. The sensor reacted in terms of resonance frequency shifts based on viscosity changes. This happens due to Staphylococcus aureus (S. aureus) growth and hence the sensor undergoes sensing of the spoiled milk. The sensitivity of the sensor was achieved well in milk than in the culture medium, as milk is a more viscous medium than the culture medium itself. Passive sensors in particular do not require internal battery as they are powered by query-field. These sensors get deformed mechanically when they are subjected to a magnetic field, which eventually launches elastic waves, within the sensor, having the highest magnitude at mechanical resonance frequency of the sensor (Ruan, Zeng and Grimes, 2003).

Subsequently, the mechanical deformation of the sensor generates a certain amount of magnetic flux that can be detected remotely by a pickup coil, i.e. without any physical contact between sensor and the apparatus, sensing takes place. Guntupalli et al. (2007) developed such a sensor for microorganisms based upon antigen–antibody interaction.

2.3.6 Photonic Sensors

Figure 2.10 Optical Sensor configuration for Microlitre Analysis of Milk

Optical capillaries have been employed as the components of photonic sensor micro-systems in the past (Borecki *et al.*, 2008).

The classical optical test methods make the overall system expensive because of the fact that fresh milk micelles have such sizes that scattering set-ups require sources and detectors that operate at the value of wavelengths $\lambda < 300$ nm, which is on the deep UV range boundary and covers the VIS range (Dress *et al.*, 1998). Figure 2.10, on the other hand, shows sensor configuration as used by Borecki *et al.* (2009).

It used low-cost optical capillaries to test the quality of milk, which required an optical sensor configuration with fibre optical set-up. This work, determined milk quality by observing its behaviour under specific heating conditions using a simple photonic system with optical capillaries. It was shown that the optical capillary is a suitable medium for analysing liquids encompassing high scattering of light, e.g. milk. Good and poor quality milk resulting from storage conditions was distinguished by this method.

While this method has the potential to be a platform for real-time milk quality classification, the overall set-up used in that is still more complex than the proposed technique based on a microwave sensor in this thesis. Despite that work claiming it to be cheaper than the other similar work involving high quality fibre, such instrumentation is relatively costlier, compared with the proposed work here, as it comprises of a light source, fibre optical link, sensing head, and two photo-detection units, which are controlled by an intelligent detection-and-control system, built based on the Atmel Co. AVR® microprocessor.

The focus of this work, primarily, is on the variation in quality resulting from defined storage conditions. Notably, this process requires the heating of milk, as the test takes into account the influence of temperature on the structure of milk, and hence local heating to the capillary is introduced by Borecki et al. (2008), which results in quite a large instrument set-up.

Table 2.1, on the following page, summarises the milk quality control techniques, in industries and the research domain, as discussed in this chapter, comparatively with their main characteristics. The table reflects back upon the current state of the art, for the milk quality monitoring, with the last row showing the position of the proposed microwave sensing technology.

Table 2.1 Comparison of Current State of the Art with Proposed Technique

Sensing/Sensor Types	Detects	Accuracy	Advantage	Cost	Limitation
pH Indicators	Acidity Changes	Variable at different levels of pH	Instant	450-750 £	Unspoiled milk pH; still good for some bacteria
Magneto-elastic Sensors	Viscosity changes due to S. aureus growth	$R^2 > 0.95$	Disposable; Wireless; Low Cost	~ 0.25 £/m of Metglas® Alloy Ribbon	≈ 18 hours Required
Gas-Sensor Arrays	Strains/Oxide Based Volatile Compounds	Variable for each Bacterium type Tested	Instant for given Threshold	3800 – 75,300 £*	Costly and Difficult to Use
IR / NIR Spectroscopy	Metabolic By-products	$R^2 > 0.90$	≈ 4 Minutes	Up to 35,000 £	Very Expensive Set-up
Amperometric Sensing	Change in Direct Current	$R^2 > 0.90$	Relatively Less Complex	1150 - 3800 £	Up to 2 Hours; Supervision Required
Optical Sensors	Scattered Light through Optical Capillaries	$R^2 > 0.95$; When Heating Conditions Maintained	Time Consuming	> 3800 £	Heating Condition Required
Protein Analysis Methods	Protein Levels	$R^2 > 0.95$	≈ 4 Minutes (Dumas)	Up to 3800 £	Technical Complexity
Fat Analysis Methods	Fat Levels	Rose-Gottlieb > Gerber Tests	Can Separate Fat Types	~ 3000 £	Steps with Reagents Required
Chromatography Techniques	Fat / Protein Extraction / Adulteration	$R^2 > 0.95$	Few ms to few minutes	Up to 60,000 £	High Expense
*Microwave Spectroscopy **(**Proposed Technology**)***	*Composition Spoilage Adulteration*	*$R^2 \geq 0.95$*	*< 1 minute*	*50 £ - 1250 £*	*Not tested for specific bacterium*

* This will decrease with the advancement in Conducting Polymers in coming years

The microwave sensor design and its construction are cheaper compared to all other types of sensing technology, discussed here, except for the magneto-elastic sensors overall. With added support of portable or handheld VNAs, it has become further possible to reduce the operational costs of the proposed microwave sensing technology down to a lower minimum.

2.4 Techniques used to Determine Milk Adulteration

In this section, an analytical review of the current state of the art is made based on methods used for detection of urea, as the main adulterant, in milk. Urea, which is the predominant component of non-protein nitrogen (NPN) present in milk, can be naturally available in milk up to 700 ppm (i.e. 70mg per 100ml). The range of urea presence, in milk, is normally between 0.01 - 0.06 %. This, combined with the protein concentration within milk, is considered an important factor in regulation of livestock feeding. Hence, this is another important reason why urea concentration should be monitored on a frequent regular basis.

Lars Nygaard, Torben Lapp and Börkur Arnvidarson (1993), secured a US patent for their urea determination method, using infrared (IR) light measurements, with a condition of milk samples having a minimum of 1% fat, dissolved lactose, and protein respectively each.

Primarily used technologies, for the detection of urea in milk, can be categorised into two types – direct, such as colorimetric, and indirect, such as degradative procedures, besides point-of-care and other methods for urea determination (Francis, Lewis and Lim, 2002); the authors conclude that urease-catalysed hydrolysis primarily dominates the chemistry behind determination of urea within clinical samples. Because the instrument is more likely to be used by non-technically trained staff, the focus has shifted to simpler procedures and data handling.

Muir and Sweetsur, (1976) have concluded that urea levels that naturally occur in bulk milk play a vital role in heat stability while contributing largely towards its fluctuations. This conventional method for urea estimation within milk uses conversion from urea to ammonia by urease, which is then subjected to colorimetric determination.

Despite the method being sensitive and precise, it requires specific urease purity which is difficult to be procured for all laboratories or dairy plants (Bhavadasan, Rajput and Ganguli, 1982); the researchers further summarised that the urea level in cows' milk was relatively higher than in buffalo milk. In addition, the conventional methods being costly and tediously time consuming, they are not

effective for being implemented regularly for real-time farm management let alone in determining urea due to deliberate forms of adulteration for commercial gain.

Another approach for urea determination in milk is the differential pH method (Luzzana and Giardino, 1999). In this technique, measurements were based upon single enzymatic reaction, in the form of urease driven hydrolysis of urea that causes pH variation directly in proportion to the urea content present within the milk sample. This method, because of using reagents and buffer solutions, is an invasive method similar to the traditional spectrophotometric method that is used as the gold standard in this project to benchmark results obtained through the proposed novel technique using microwave spectroscopy, which is non-invasive.

Paradkar, Singhal and Kulkarni (2000) developed a detection technique for externally added urea under the malpractice of making 'synthetic milk' which is made up using a mixture of easily available and low cost ingredients such as – urea ($CO(NH_2)_2$), water (H_2O), sodium chloride (NaCl), sucrose ($C_{12}H_{22}O_{11}$), sodium bicarbonate ($NaHCO_3$), detergent and vegetable oil. The protocols suggested by the researchers were also similar to the conventional invasive approach with neutralised pH 7.0 buffer milk reagents while using soybean urease.

Each of these listed ingredients plays a specific role in order to make the product, which looks, feels and tastes like milk; moreover, adulteration of original milk with such synthetic milk up to 5% is not detected at all under tests like lactometer reading or alkalinity due to the aforementioned similarity. Urea, in particular, plays a role to contribute towards milk nitrogen.

Urea is also known to be externally added to improve milk's heat stability. The Potentiometric approach, has been adopted by researchers in the past, to enable determination of a wider spectrum of ions and to allow for inexpensive and portable equipment, using NH_4^+ ion sensitive electrode under Double Matrix Membrane technology acting as the transducer with a layer of immobilised urease (Trivedi *et al.*, 2009).

The disposable biosensor was designed using thick film screen-printing with Ag/AgCl reference electrode. Lima, Fernandes and Rangel, (2004) constructed a versatile method that could simultaneously be used either as spectrophotometric detection or conductometric detection of urea. A thermos-reactor was used to hydrolyse urea enzymatically and convert it into ammonium (NH_4^+), which eventually forms Ammonia (NH_3) by merging with an alkaline solution. In conductometric detection, Ammonia modifies conductance of Boric acid (BH_3O_3) solution whereas in spectrophotometric detection it changes the colour of the bromothymol blue (BTB) indicator. This sequential injection technique, although it achieves full automation, is very technically complex to set-up like the other conventional approaches listed above.

Renny *et al.* (2005) developed a piezo-electric sensor based on an enzyme that measures gas pressure evolving within the sample under test, with liquid to gas ratio value 1:2.5 giving optimum results. The most current state of the art from conductometric, optical, thermometric and potentiometric methods are too delicate for application without extensive pre-treatment, especially with raw milk (Nikoleli, Nikolelis and Methenitis, 2010). On the other hand, the traditional methods of analysis, though precise, are very time consuming and mainly laboratory bound.

Table 2.2, on the following page, summarises further attributes of the current state of the art for the design considerations of this research work. It shows primary characteristic features of the standard existing practices in industrial as well as research domain besides that of the proposed technique. The last row in the table shows the status of the proposed technique for each of the listed attributes.

The proposed technology is non-ionising, non-destructive, low profile, portable and rapid measurement technique, the features that no other currently in use method possess in a single system. In this research project the proposed microwave sensing technique is tested for detection of spoilage of milk, milk type classification based on its fat composition, i.e. skimmed-milk, semi-skimmed milk, and whole milk, and urea adulteration of the milk besides detection of detergents in deionised water.

Table 2.2 Milk Quality Testing Techniques with their Attributes and Applications

Technique [a]	Portable	Non-Invasive	Non-Destructive	Real Time	Application / Production Stage	Further References
pH Level Sensing	Yes	No	Yes	Yes	Spoilage Detection/ Dairy and End User	(Santos *et al.*, 2003; Fromm and Boor, 2004; Helland, Wicklund and Narvhus, 2004)
Magnetoelastic Remote-Query Sensing	Yes	No	Yes	Yes	Spoilage Detection (Tested for S. aureus)/ End User	(Winquist *et al.*, 1998; Di Natale *et al.*, 2000; Puckett *et al.*, 2003; Cai *et al.*, 2004)
Gas-Sensing Arrays	Yes	No	No	No	Shelf-life Detection/ End User	(Compagnone *et al.*, 2015; Anand and Sridhar, 2018)
IR/NIR Spectroscopy	No [b]	Yes	Yes	Yes	Classification + Spoilage Detection/ Processing at Dairy and End User	(Ellis *et al.*, 2002; Al-Holy *et al.*, 2006; Nicolaou, Xu and Goodacre, 2010)
Amperometric Sensing	No	No	No	No	Current Change (Electro-chemical)/ End User	(Atherton and Newlander, 1977; Nascimento *et al.*, 2017)
Photonic Sensing	No	No	No	No	Scattering of Light (Spoilage)/ End User	(Romaniuk and Dorosz, 2006; Das, Goswami and Biswas, 2016)
Kjeldahl Method / Dumas Method	No	No	No	Dumas (Yes) Kjeldahl (No)	Protein Composition Analysis/ Processing at Dairy	(Hantsis-Zacharov and Halpern, 2007; Fox, 2011a)
Gerber Test / Babcock Test / Rose-Gottlieb	No	No	No	No	Fat Composition Analysis/ Processing at Dairy	(Matheson and Otten, 1999)
Chromatography Techniques	No	No	No	Yes	Extraction and Separation/ End User	(Dai *et al.*, 2010; Nascimento *et al.*, 2017)
Spectro-Photometry	No	No	No	No	Urea Adulteration/ End User	(Francis, Lewis and Lim, 2002; Lima, Fernandes and Rangel, 2004)
Microwave Spectroscopy	*Yes*	*Yes*	*Yes*	*Yes*	*Composition Spoilage Adulteration / Could be used at all stages*	*Proposed Technique*

[a] Current State of the art [b] Portable devices being further researched

2.5 Summary

Based on the limitations of current techniques, there is a need for further development. This research project, attempts to address these limitations by the design and development of a novel sensory system that is able to distinguish among given types of milk, based on composition, spoilage, and contamination ensuring milk quality monitoring in a non-invasive, non-destructive, real-time and reliable manner.

When it is hard to overcome all the limitations in all the characteristics as shown, there is still a way to minimize the overall shortcomings of existing approaches. From the above discussion, it is clear that there is scope and need for improvement in milk quality testing methods.

Thus, this research work is an attempt to address the aforementioned limitations of existing technologies in a less complex, non-invasive, non-destructive, real-time, portable yet reliable manner by investigating the use of microwave sensor technology.

CHAPTER 3

EM WAVES AND MICROWAVE WAVE SENSORS

3.1 Background

Microwave spectroscopy was broadly defined as “the study of interaction between matter and radio waves of wavelengths (λ) between a few metres to a few tenths of a millimetre” (Townes, 1952). It was introduced, at large, first with experiments related to dielectric properties of matter using Hertzian waves. The use of microwave spectroscopy has become prominent since World War II with radar and other technical developments in microwave generation of techniques. Subsequently this technique has been used for a wide variety and broad range of applications, e.g. food quality, health monitoring, drug detection, water pollutants etc. (see section 3.5).

This technique is similar to IR spectroscopy at large with the main difference being significantly lower operational frequencies, hence lower power, as compared to IR spectrum and additional flexibility of low-profile planar sensors besides other cavity sensors as discussed in this chapter. Figure 3.1 illustrates the full EM wave spectrum with the frequency and wavelengths of all waves.

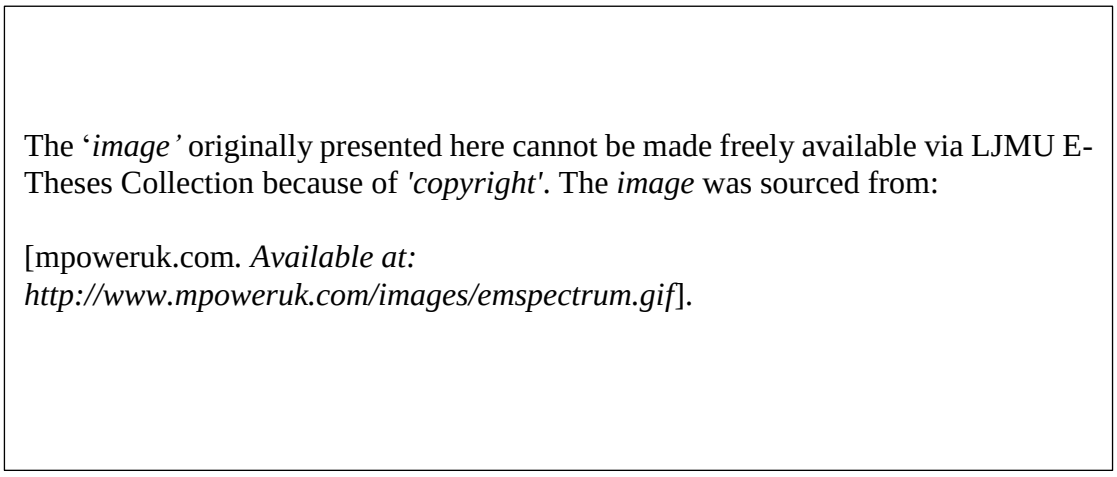


Figure 3.1 Full EM Wave Spectrum with wavelengths and frequencies

The EM wave spectrum covers the full span of non-visible as well as visible light signals made up of Electric and Magnetic fields perpendicular to each other and perpendicular to the direction of propagation.

3.2 Working Principle

The primary principle of these sensors that operate at microwave or radio frequencies (RF) range is based on the interaction of signals with the medium of the material sample being tested. This interaction between microwave signal and the medium material can be in the form of amplitude attenuation or phase shift, and it determines the relative permittivity and permeability (Alhajeri, 2010). Figure 3.2 illustrates the experimental setup involving microwave sensor in form of a microwave resonator cavity connected with a vector network analyser to enable and record sensing measurements.

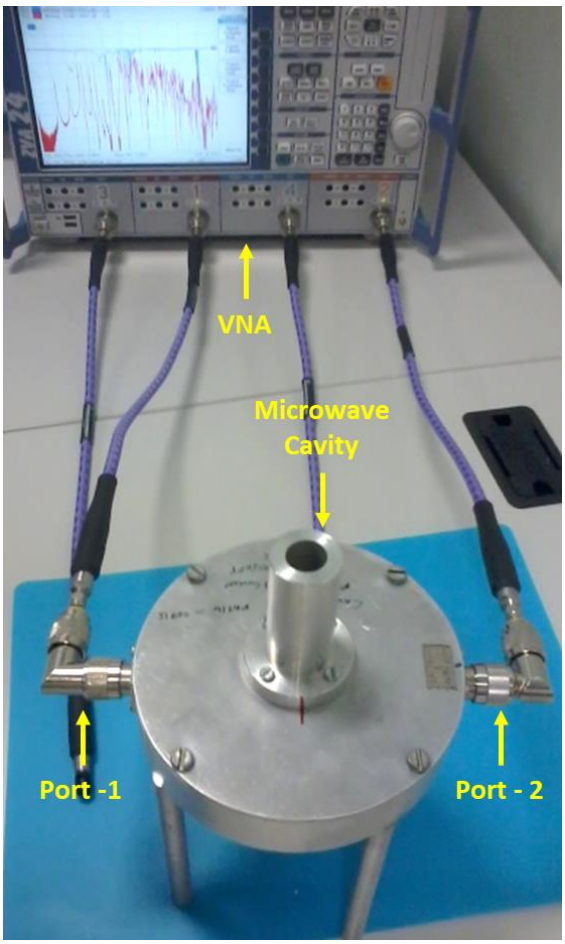


Figure 3.2 Experimental setup for Microwave Sensing explaining its Working Principle

Microwave sensing (EM wave sensors) operate based upon the fact that the material under test condition, e.g. milk sample in this case, when placed into proximity or in direct contact with a microwave sensor, interacts with the EM waves that can be specifically correlated with the properties of the given material (milk). Particularly, the sensing is based on the interaction of propagating or resonating modes with the liquid under test.

3.3 Advantage of Microwave Sensors

The primary advantage of using microwave sensors is that they can be implemented for a wide range of applications in a non-destructive, cheap yet effective manner, while being able to measure non-invasively from a short distance, due to penetrating waves without creating any health hazards (Bjarnadottir et al., 2015).

These types of sensors are working on non-ionising radiation meaning that the EM wave radiation that does not contain enough per photon energy to ionize atoms (or molecules) to be able to completely remove an electron from that atom (or molecule). This implies that these types of sensors cause no health hazards to the human body or animals especially when used at lower frequencies up to few tens of GHz. In addition, they can be easily retrofitted to an existing industrial set-up.

3.4 The Wave Equations

The three basic material properties are described by conductivity (σ), permittivity (ε), and permeability (μ). Here, dielectric permittivity (ε) measured in Farads/meter (F/m), and magnetic permeability (μ) measured in Henries/meter (H/m), are complex quantities.

Two of the total four Maxwell equations give the relationships between electric field vector, $\bar{E}$, and magnetic field vector, $\bar{H}$:

$$\bar{\nabla} \times \bar{H} = \sigma \bar{E} + \varepsilon \frac{\partial \bar{E}}{\partial t} \tag{3.1}$$

$$\bar{\nabla} \times \bar{E} = -\mu \frac{\partial \bar{H}}{\partial t} \quad (3.2)$$

The above two equations, eq. (3.1) and eq. (3.2), are Ampere's law and Faraday's law, which relate time variations of one field to spatial variations of the other, i.e., they state that time-varying electric fields generate magnetic fields, and time-varying magnetic fields generate electric fields, respectively (Mehdizadeh, 2015c).

Furthermore, these laws show that the ratio between the intensities of electric field and magnetic field in time and space are set by surrounding material properties. For example, a vacuum surrounding would have permittivity and permeability, but not conductivity because there are no free charges in a vacuum space.

Now if we consider the steady-state time-varying systems only then it further simplifies (is reduced to) the relation between $\bar{E}$ field and $\bar{H}$ field, from instantaneous values of fields, to only corresponding values of fields at a given operating frequency. Then equations eq. (3.1) and (3.2) can be rewritten, respectively, as:

$$\bar{\nabla} \times \bar{H} = \sigma \bar{E} + j\omega \varepsilon \bar{E} \quad (3.3)$$

$$\bar{\nabla} \times \bar{E} = -j\omega \mu \bar{H} \quad (3.4)$$

Where $\omega = 2\pi f$ is angular frequency in radians/second and f is the frequency in Hertz (Hz). The values of permittivity and permeability in free space are, respectively, $\varepsilon_0 = 8.85 \times 10^{-12}\ F/m$, and $\mu_0 = 4\pi \times 10^{-7}\ H/m$, and $j = \sqrt{-1}$ is complex operator.

Combining equations eq.(3.3) and (3.4) gives the following useful equation for solving practical boundary value problems such as those in probes and sensors because each of the following is a now a differential equation with only one of the field type as a function of spatial dimensions.

$$\nabla^2 E + k^2 E = 0 \quad (3.5)$$

$$\nabla^2 H + k^2 H = 0 \quad (3.6)$$

Here, the operator (∇^2) is called Laplacian and k is the wave number, $k = \omega\sqrt{\mu\varepsilon}$.

Solving these equations, combined with a given set of boundary conditions help determining the propagation modes, which is nothing but a form of wave (and hence one form of wave function) able to travel through a given microwave device.

3.5 Existing Microwave Sensors

In Chapter 2, current techniques and recent advancements in milk quality testing for spoilage detection and adulteration of milk were reviewed and critically analysed. In this section, the current state of the art of microwave sensors is briefly discussed.

Microwave sensors have been used for a wide variety of applications and based on their application, they are mainly categorised into two groups. One group is for applications related to distance, movement or shape measurement comprising of radiometer, topographic or radar sensors (Alhajeri, 2010). The second and the largest group of applications, which is also related to this research work, is of the sensors like microwave resonance cavity, waveguides and transmission or reflection sensors, aimed at the measurement of material properties, specifically those of liquids and gases flowing through or held within them (Nyfors, 2000).

Figure 3.3 shows the frequency band particularly used for various applications of microwave based studies. International Telecommunication Union (ITU) defines the microwave band of frequencies that varies between 300 MHz to 300 GHz. From this broad range of spectrum, the microwave sensing applications take place between a few megahertz [MHz] to tens of gigahertz [GHz], in which part of the electromagnetic wave spectrum, the most wireless telecommunication devices are also accommodated (Mehdizadeh, 2015c).

These sensors are basically converters of electrical signals to electromagnetic fields, or vice versa and the whole microwave sensing system comprises of the sensor, the material being interrogated or processed with the field that is imposed on it and the electrical signal or power. When the material is being interrogated, the device is called a “probe” or a “sensor” and when significant energy is imparted to the material, it is called an “applicator” (Mehdizadeh, 2015b).

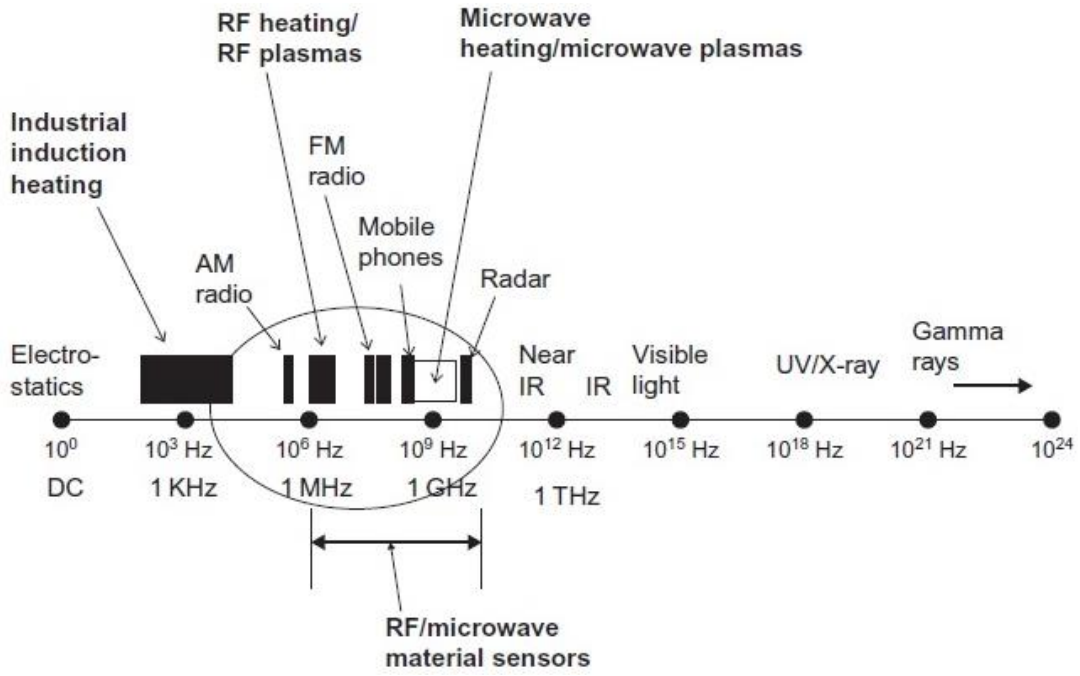


Figure 3.3 Range of frequencies for Microwave Applications

(Source: Mehdizadeh, 2015c**)**

Following are the various types of microwave sensors in sensing applications currently. The microwave sensors discussed below are near-field, which means, having the distances between the resonator and the material (under test) smaller as compared with the wavelength of microwaves.

3.5.1 Waveguides

A waveguide is a hollow metallic structure that guides microwaves (or other wave types such as sound), with the aim of minimal loss of energy by restricting wave expansion to one to two dimensions. A waveguide has propagation modes which is one solution of the wave equations, or, in other words, the form of the wave (Emerson, 2002). Because of the constraints of the boundary conditions, there are only limited frequencies and forms for the wave function, which can propagate in the waveguide.

The lowest frequency in which a certain mode can propagate through the waveguide is referred to as the cut-off frequency of that mode. In addition, the mode with the lowest cut-off frequency is identified as the fundamental mode of the given waveguide, and that lowest cut-off frequency becomes the waveguide cut-off frequency.

Figure 3.4 shows a standard small size rectangular shape microwave waveguide. There are several other forms of waveguide being used and tested in various fields too.

Propagation modes are calculated by solving a formula called Helmholtz equation along with a set of boundary conditions based up on the geometrical shape and wall materials bounding the region. Eq. (3.5) and Eq. (3.6) are the forms of the Helmholtz equation where the operator (∇^2), is called Laplacian.

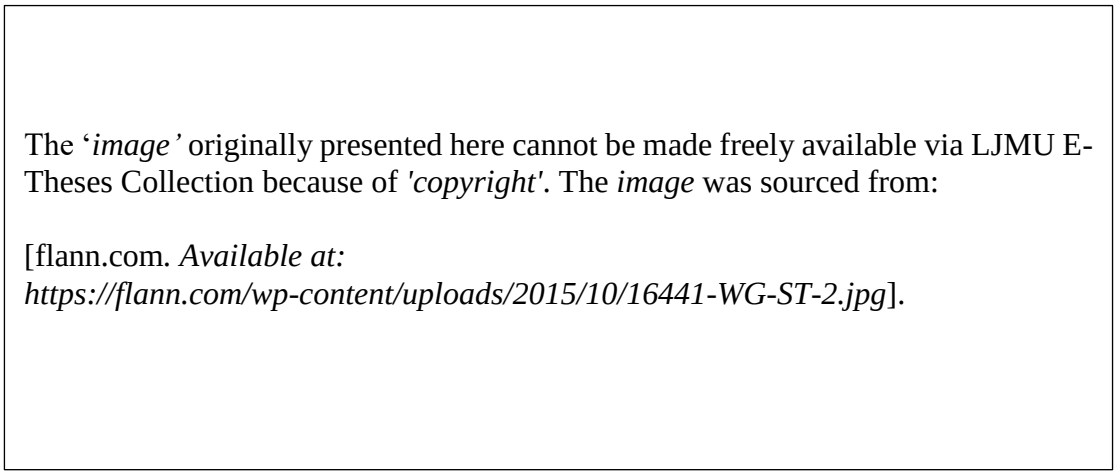


Figure 3.4 A small Rectangular Waveguide

These types of microwave devices are used for guided wave measurements of dielectric property tests of material, which are normally placed inside the waveguide and spectral signatures recorded for further analysis of the data.

3.5.2 Cavity Resonators

Microwave cavities are available in several shapes and forms. Generally, they are nothing but voids mainly enclosed by high-conductivity metal walls. The stimulus frequency when coupled into the microwave cavity will set up standing waves only if the stimulus frequency matches one of the natural resonant frequencies of the cavity as shown in Figure 3.5, (Mehdizadeh, 2015a).

This phenomenon is similar to the propagation modes as discussed in the case of microwave waveguides in section 3.5.1.

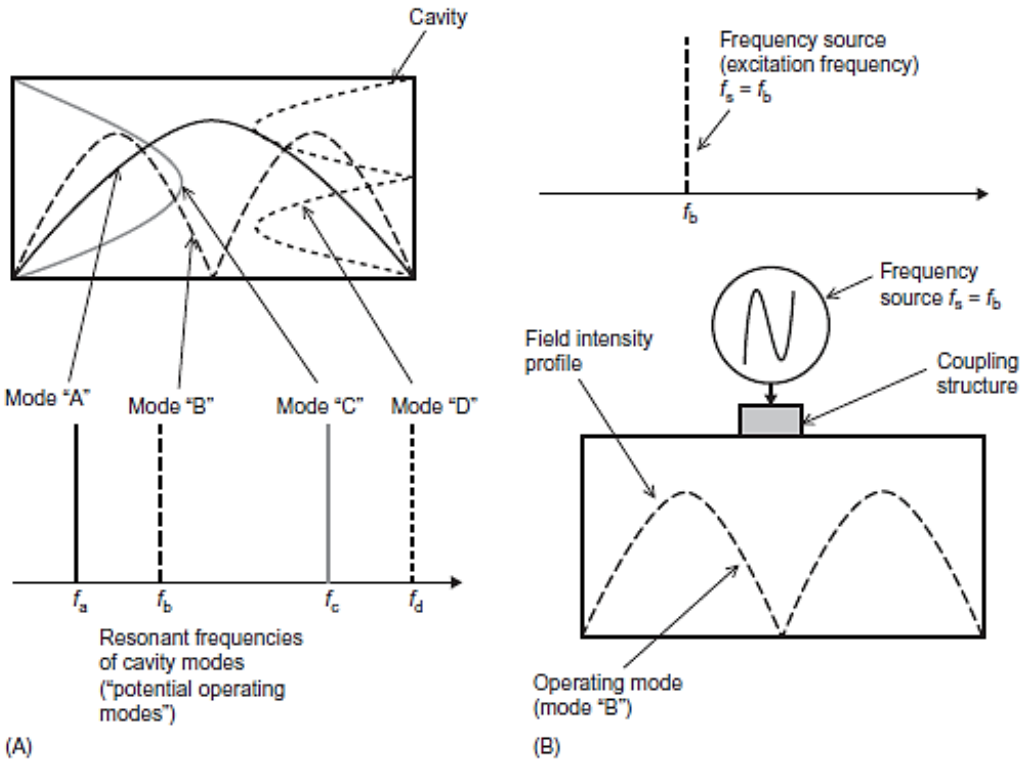


Figure 3.5 Modes in microwave cavity: (A) All modes are potential operating modes; (B) Operating mode is the one that matches the excitation frequency

(Source: Mehdizadeh, 2015a**)**

A variety of novel applications for this type of sensor have been made before in various domains such as water-in-oil measurements (Sharma, Lao and Falcone, 2018) for vegetable oils (Osman *et al.*, 2014) etc. Figure 3.6 shows a microwave resonator cavity sensor with a milk sample under test.

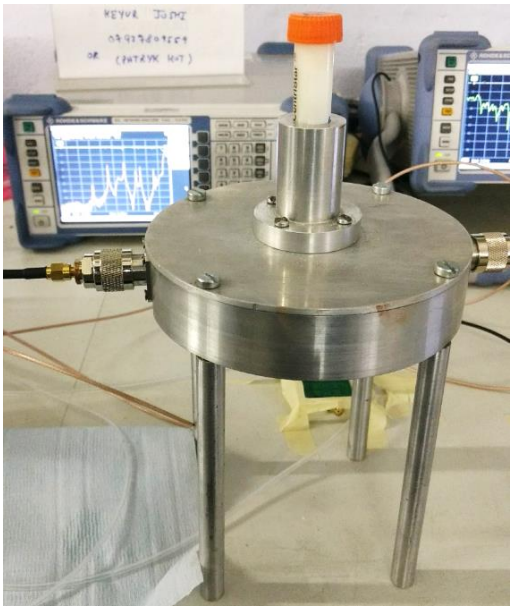


Figure 3.6 A microwave resonator cavity testing milk sample

3.5.3 Interdigitated Electrodes (IDEs)

These types of sensors offer several advantages, such as working with low volumes of samples in an electrochemical sensing. The electrodes also known as “fingers”, in this type of configuration, typically enhance sensitivity and detection limits by allowing a small size planar sensor device effectively of up to 1 to 2 cm size in width and length and a few mm in thickness.

Interdigitated structures are typically used for capacitive transduction; the interdigitated electrodes effectively improve the overall capacitance of the device. When exposed to the sensing material under test such as milk, there is some change expected in the capacitance of the planar IDE sensor, which is eventually recorded in resonance frequency changes as the C of the IDE structure changes. These types of sensor only allow access from one end, i.e., 1-port configuration, which is particularly useful when the access to a material under test (MUT) is limited or the other side should be open to the ambient (Rivadeneyra *et al.*, 2016).

Figure 3.7 shows an IDE sensor with Gold coated electrodes (fingers) and fitted “well” to contain samples of up to 500µl size for invasive sensing and detection. It has several applications including surface notch-type damage detection in metals (Li *et al.*, 2018), and in healthcare monitoring for detection of specific biomarkers in human cerebrospinal fluid (Fok *et al.*, 2015) as further explained in sub-section 4.4.2, under microwave sensor design.

Figure 3.7 An IDE Sensor with 9x9 interdigitated electrodes fitted with well

3.5.4 Planar Resonators

Planar resonators are widely used in applications where portability and low-profile characteristics are a requirement for compact overall size in limited space. Several applications of these types of sensors have been made in the past in various domains and they are available in several forms such as split ring resonators (Choi *et al.*, 2015; Mohd Bahar *et al.*, 2017), microstrip-resonator (Zarifi, Thundat and Daneshmand, 2015), hairpin resonators (Huang, Liu and Chen, 2017; Liu *et al.*, 2018) and many other forms.

Figure 3.8 A 2-port Microstrip Planar Resonator Sensor

(© 2017 IEEE. Reprinted, with permission, from Mohd Bahar, A. A. *et al.*, High-efficiency microwave planar resonator sensor based on bridge split ring topology, *IEEE Microwave and Wireless Components Letters***,** June 2017**)**

Figure 3.8 shows a Microwave Planar Sensor based on Bridge Multiple Split Ring Resonator Topology (BMSRRT) based planar hairpin resonator, which behaves as a fluidic sensor (Mohd Bahar *et al.*, 2017).

3.5.5 Comparison of Microwave Measurement Methods

Table 3.1 A simple comparison for microwave measurement techniques

The '*table'* originally presented here cannot be made freely available via LJMU E-Theses Collection because of *'copyright'*. The *table* was sourced table 1.1 from:

[*Li, X. and Jiang, Y. (2010) Design of a Cylindrical Cavity Resonator for Measurements of Electrical Properties of Dielectric Materials, radio center Glave university. Gavle University. Available at: http://urn.kb.se/resolve?urn=urn:nbn:se:hig:diva-7687*].

Table 3.1 is the brief comparison of standard dielectric and magnetic measurement techniques based on microwave sensing, as given by Li and Jiang, (2010). As the table depicts, the capacitor method obtains complex dielectric permittivity by measuring the change of capacitance and of conductance due to the device with and without specimen. Transmission line method is simpler due to not requiring any specific devices for the measurements but its sample preparation is complicated, as it needs to be in an annular geometry or slab.

The cavity resonator methods generally takes the form of a cylinder and hence the sample contained for the measurements is held in test-tube of a predefined size and shape depending upon the structure of cavity in use. The open resonator methods are available in the form of hemispherical or spherical resonators to test thin film materials. Whereas, the free space methods use two antennas for transmission and reception when the material under test is left on a fixed slab normally.

From the observations made above and the techniques reviewed, the design considerations for this project are explained, in section 3.6. The considerations are made based on the currently used sensor types to choose a suitable microwave sensor design, to enable real-time milk quality testing attempting to resolve the limitation of existing standards by highlighting the key areas where further development can take place.

3.6 Sensor Design Considerations: For Milk Quality Monitoring

3.6.1 Primary Design Considerations

Before commencing the sensor designing part for microwave sensing application in milk quality monitoring, it was essential that a specific set of design considerations be laid out to address the requirement of the project as well as to satisfy the need of industry. Here, in this section all the design parameters are discussed to enable microwave sensor design. The sensor design constraints included the criteria to eliminate the limitations of currently existing techniques as previously discussed in Chapter 2.

The microwave sensor system should be low profile, i.e. portable and compact with lightweight and dimensions no more than few cm sizes in length and width, and hence not limited to industrial setup. Furthermore, it should be non-destructive and non-invasive in application, eliminating the requirement of several stages of preparing reagents and adding them to actual milk product under test, unlike other traditional methods, making it resource-efficient technique.

Therefore, the microwave sensor technology can also be accessible outside the laboratory premises by avoiding involvement of hazardous chemicals. Additionally, the final sensor design should be low-cost as compared to other existing techniques while enabling rapid measurements making it applicable for a time-efficient quality monitoring system.

3.6.2 Identifying the Frequencies of Interest

Based on above primary considerations, as explained in section 3.5, from a broad range of frequencies between a few megahertz [MHz] to tens of gigahertz [GHz] where the microwave sensing applications are housed, frequencies of interest for this project were narrowed down and identified between approximately 2 GHz up to 8 GHz.

This consideration was made to eliminate lower end frequencies to minimise any possible low frequency, low power noise signals considering several telecommunication applications already taking place in lower frequencies than this.

Moreover, it was important to accommodate two key Industrial, Scientific and Medical (ISM) bands defined, at 2.400 - 2.500 GHz and 5.725 - 5.875 GHz, by ITU as “Operation of equipment or appliances designed to generate and use local radio frequency energy for industrial, scientific, medical, domestic or similar purposes, excluding applications in the field of telecommunications” (International Telecommunication Union, 2016).

This was chosen particularly due to the possibility of extending this scientific work to an industry oriented sensor system. The ISM bands are defined by the ITU Radio Regulations No. 5.138, 5.150, and 5.280, following world radio conferences.

3.7 Summary

To develop an overall quality testing system for packaged milk types, working principle and advantages of EM wave sensing techniques were investigated along the study of various near-field microwave sensors. Based on the observations made from literature and current state-of-the-art, design constraints for the proposed microwave sensor system were established in this chapter.

The primary focus of milk quality monitoring was established on overall comparative measures of composition, spoilage detection and contamination check, which represent the qualitative measures of the milk and not specifically the quantitative measurements; as there are rigorous procedures, already available, for industrial use for exact quantifiable composition check for milk such as, for example, Chromatography techniques.

The following chapter explains dielectric property of milk and addresses the designing of available microwave sensors, namely resonator cavity and IDE sensor, with their simulations, and further development of a fluidic planar resonator sensor to suit the design considerations made in this chapter. It should be noted that waveguide mainly suitable for solid material property tests, was not considered for the design, simulation and actual test measurements, and instead the single probe analysis, for milk samples, was preferred.

CHAPTER 4

DIELECTRIC PROPERTY OF COW MILK AND SENSOR DESIGN

4.1 Dielectric properties of cow milk

From the discussions made ahead, it is clear that the development of a new technique, which can assess quality of cow milk using microwave spectroscopy, could benefit from having the required understanding of dielectric properties of such milk types. In the literature reviewed, it was found that the dielectric constants for raw milk (with 100 % concentration of milk) and diluted milk (with 70 % milk concentration to deionized water), both, decrease with increasing frequency.

This phenomenon was identified to occur more at the lower frequency range of 10–4500 MHz but this decrement in values for dielectric constant for raw milk was more rapid than in the case of diluted milk, at room temperature of 22 °C (Guo *et al.*, 2010). For raw milk, these values of decrement were 97.7, 68.1, and 65.9, whereas for 70%, diluted, milk solution the same were 93.3, 70.9 and 69.1, at 10 MHz, 915 MHz, and 2450 MHz of frequencies, respectively. Another potential indicator for predicting milk concentration, and freshness, is its dielectric loss factor. The researchers further observed that because the adulteration of milk with water dilutes the overall milk concentration, ionically, the loss factor tends to decrease with the increased amount of water content, i.e. decreasing milk concentration.

Researchers in another similar work concluded that, for cow's milk, its protein content as well as the temperature of milk affect both the values of dielectric constant and the loss factor. The frequency of electric field also influences these two values. Within the range of 10–4500 MHz, the dielectric constant decreased with increasing frequency (Zhu *et al.*, 2015).

The value of dielectric loss factor increased with increasing temperature below frequency values of around 600 MHz and decreased above 1000 MHz of frequency values. A poor correlation was observed between the amount of protein content and dielectric constant in the region of 100–600 MHz frequency values. Based on these dielectric constant values, it meant that this frequency range was insufficient

for being used to develop a milk protein detector. The dielectric property of milk was determined and analysed for all three milk types, in this work as explained in the section 4.2.

4.2 Measurement of Dielectric Property of Cow Milk

Materials can be classified according to their complex-valued dielectric permittivity (ε), upon comparison of their real (ε′) and imaginary (ε″) components. To understand the dielectric property characteristics of milk the milk type, i.e., with respect to their fat components, dielectric property single probe tests were carried out.

We use Eq. (3.3) and the fact that current density J (A/m^2) is related to the magnetic field by:

$$\bar{J} = (\sigma + j\omega\varepsilon)\bar{E} \tag{4.1}$$

The Eq. (4.1) shows that the imposition of an electric field into a material will produce electrical currents, the amplitude and direction of which are proportional to the electric field intensity. Two components of these currents are conductivity σ in Siemens/m, which is frequency independent, and the second type of currents which are generated by the imposition of an electric field into a material are due to permittivity, represented by ε in Eq. (4.1) (Mehdizadeh, 2015c).

Permittivity itself is composed of real and imaginary components and is represented in complex algebraic format as:

$$\varepsilon = \varepsilon' - j\varepsilon'' \tag{4.2}$$

The real part of the complex permittivity ε' is responsible for phase shift of the electric field, and the imaginary part ε'' is responsible for energy losses into the material. The permittivity of dielectric materials can be expressed with that of free space as:

$$\varepsilon' = \varepsilon_0 \varepsilon_r' \tag{4.3}$$

And

$$\varepsilon'' = \varepsilon_0 \varepsilon_r'' \tag{4.4}$$

Where ε_r' and ε_r'' are called relative permittivity and loss factor (dissipation factor D_f), respectively. Relative permittivity ε_r' is also known as dielectric constant (Mehdizadeh, 2015c). The loss tangent, also known as power factor, characterizes dielectric loss properties as:

$$\tan\delta = \frac{\varepsilon_r''}{\varepsilon_r'} \tag{4.5}$$

Table 4.1 shows how the ratio of these two values is used to determine the dielectric property of the material under test (Chen, 2017).

Table 4.1 General Classification of Materials based on Permittivity

$\varepsilon_r''/\varepsilon_r'$	**Current Conduction**	**Field Propagation**
0	-	Perfect dielectric lossless medium
$\ll 1$	Low-conductivity material; Poor conductor	Low-loss medium; Good dielectric
≈ 1	Lossy conducting material	Lossy propagation medium
$\gg 1$	High-conductivity material; Good conductor	High-loss medium poor dielectric
∞	Perfect conductor	-

4.2.1 Sample Preparation

Three commercially purchased packaged milk comprised of cow milk in each category – skimmed milk, semi-skimmed milk and whole milk were tested to determine their dielectric parameters. Table 4.2 notes down the compositional elements of each milk type that was considered for the test measurements using coaxial probe analysis of dielectric property in terms of dielectric permittivity.

Table 4.2 Milk compositional values for each milk type under test

Typical Values (per 100 ml)	Milk Categories		
	Skimmed milk	Semi-skimmed milk	Whole milk
Energy (kJ)	147 (35 kcal)	209 (50 kcal)	268 (64 kcal)
Fat (g)	0.1	1.8	3.6
Saturates (g)	<0.1	1.1	2.3
Protein (g)	3.4	3.6	3.2
Carbohydrates (g)	5.0	4.8	4.7
Sugars (g)	5.0	4.8	4.7
Salt (g)	0.1	0.1	0.1
Calcium (mg)	124.0	124.0	120.0

The milk types were purchased from a high street supermarket for the longest available expiry dates and were made into four samples each. The dielectric data was measured every morning at approximately same time for Monday (Day-1), Tuesday (Day-2), Wednesday (Day-3), Thursday (Day-4), Friday (Day-5) and then the following Monday (Day-8), when the milk samples were spoiled.

4.2.2 Measurement Conditions

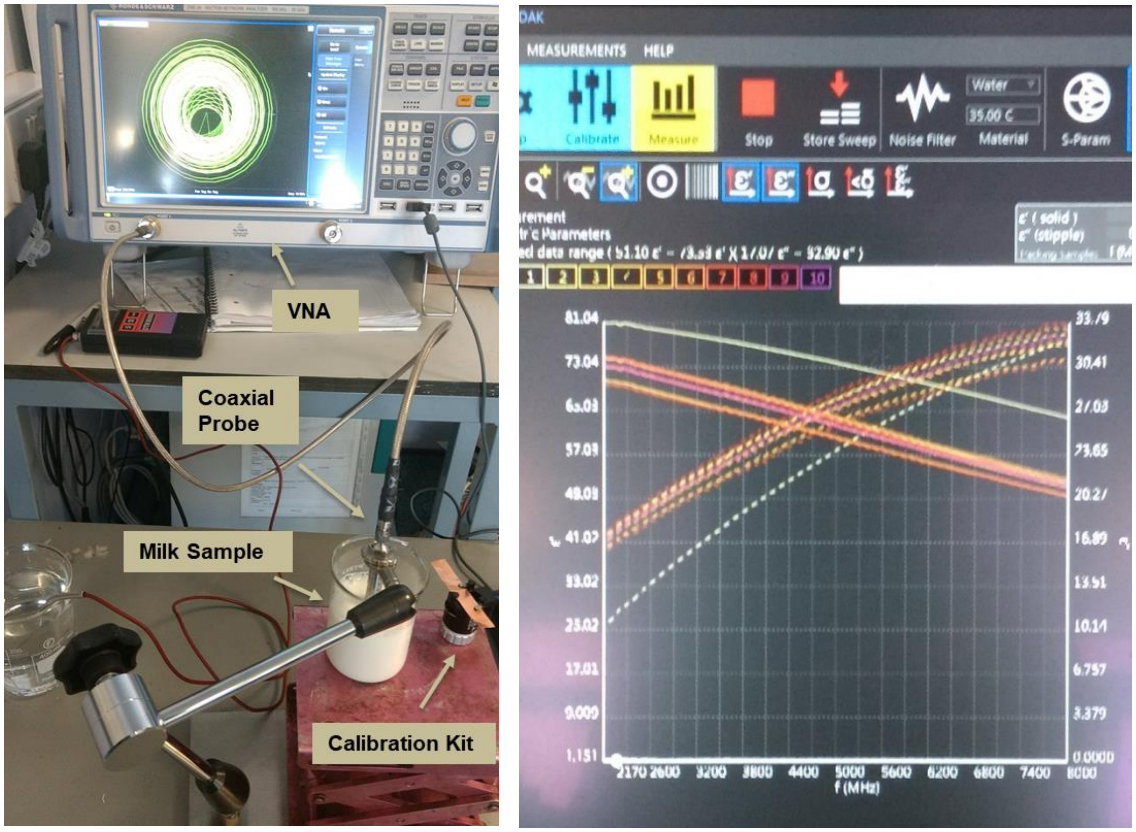
The dielectric permittivity was measured for each milk type between the frequency values 2GHz to 8GHz. All milk types were measured.

Table 4.3 Measurement conditions for Dielectric Property test of Cow Milk types

Specifications	Values
No. of Milk Types	3 Milk Types (Skim, Semi-skim, Whole) x 4 samples
Repetitions	4 times each (for 6 days)
Sample Size	≈ 250 ml (for coaxial probe)
Test Period	1 week (Day No.: 1, 2, 3, 4, 5, and 8)
Lab Temperature	13 °C ± 2 °C
Coaxial Probe	SPEAG DAK 3.5
Frequency Sweep	2 GHz – 8 GHz
VNA / Step Size	R&S® ZNB 20 100 kHz – 20 GHz / 5 MHz

Table 4.3 lists measurement specifications and conditions observed during dielectric property tests of milk. In the following sections, the experimental set-up is shown followed by a discussion of results achieved.

4.2.3 Instrumentation and Procedure



(a) (b)

Figure 4.1 (a) Dielectric Property Measurement DAK Kit,
(b) Dielectric Permittivity values for Skimmed, Semi-skimmed and Whole Milk Samples

Figure 4.1 shows the dielectric parameter, for three milk types, being tested using Speag's high precision single probe dielectric assessment kit (DAK) 3.5, calibrated with deionised water, and by using R&S® ZVL Vector Network Analyser.

4.2.4 Result and Discussion

Figure 4.2 illustrates the real component (ε′) plotted against the frequencies (f).

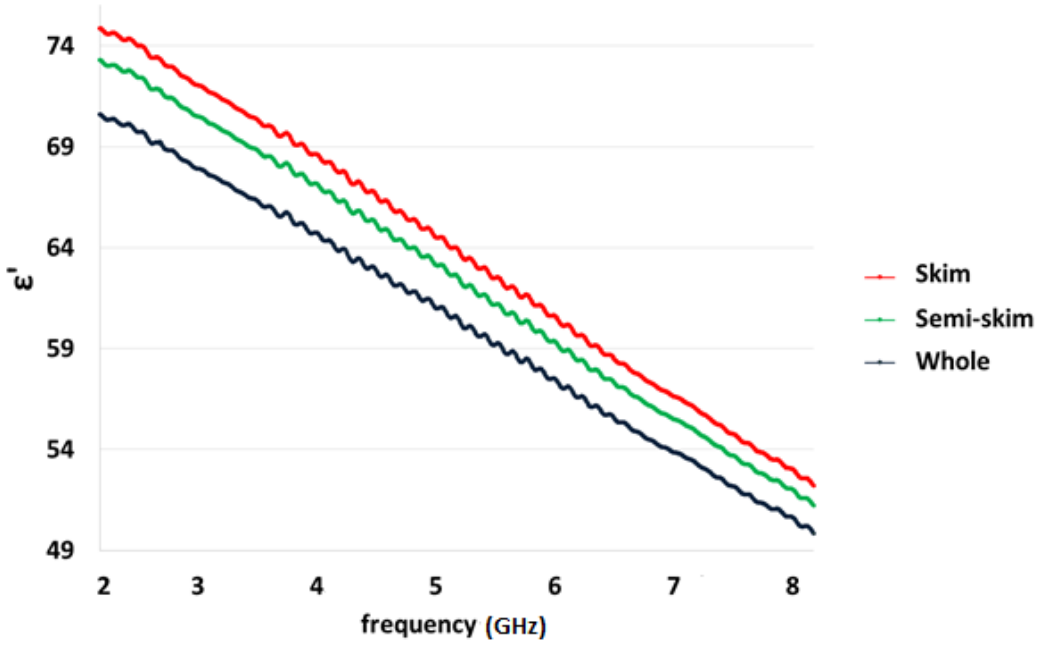


Figure 4.2 Dielectric Permittivity vs. Frequency values for three Cow Milk types

We can see the clear distinction of permittivity values among all three types of milk, due to varying fat contents. Skimmed milk with the least fat content has the highest dielectric value whereas the whole milk samples show the least dielectric value of the three types. The graphs here show the dielectric real component on Day-1 of purchase for the three milk types. The test measurements were made in a climate-controlled room with a minimum variation in temperature values.

The graph of relative permittivity vs. frequencies shows declining behaviour, i.e. the relative permittivity values decrease with increase in frequencies. Moreover, the results comply with the fact that the dielectric permittivity values are reducing as we go along from skimmed milk through semi-skimmed milk to the whole milk, with reductions in fat content; the deionised water has a higher dielectric constant value than even skimmed milk, which equals to 80.10 at 20°C, indicating it is polar.

Figure 4.3, shows the dielectric permittivity variations over an 8-day period for skimmed milk. The figures on the right show temperatures in °C, which also affect directly the results plotted in graphs. D1, D2 … D8 denote Day1, Day2 … Day8, respectively. Note that the Day-8 graph result was a complete outlier, supporting the fact that by eighth day of the tests milk samples had completely gone off, both in visible inspection and by means of smelling odour.

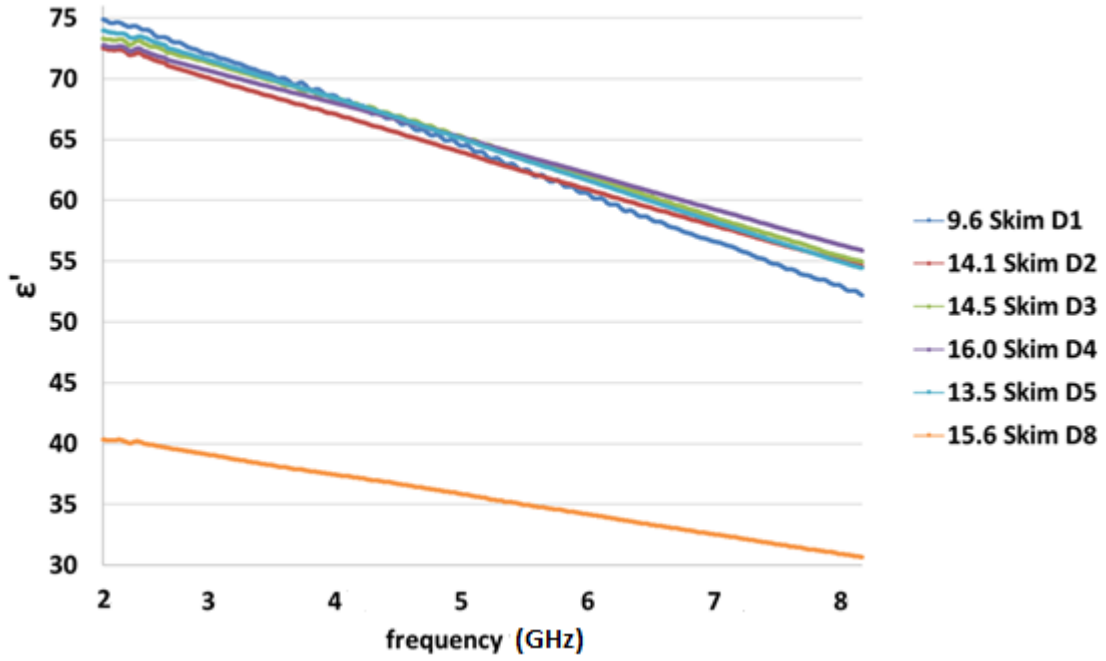


Figure 4.3 Dielectric Permittivity vs. Frequency values for Skimmed milk samples Day-1 to Day-8

Figure 4.4 illustrates the similar graphs for semi-skimmed milk with the similar trends of drop in the dielectric permittivity values with the rise in frequencies, complimenting the results achieved in earlier research work as explained in section 4.1.

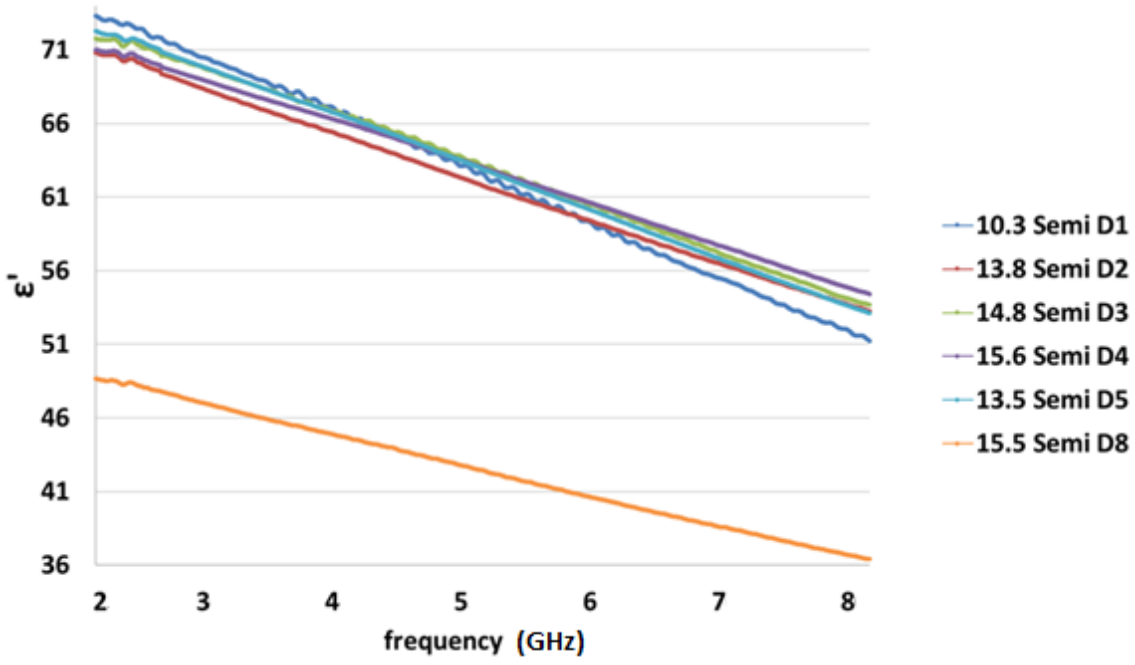


Figure 4.4 Dielectric Permittivity vs. Frequency values for Semi-skimmed milk samples Day-1 to Day-8

Numbers to the right of the graph show temperature in °C and D1 through D8 indicate days 1 through 8, respectively. Even in this case, Day-8 was an absolute outlier as compared to other five days, for the same reason of milk samples having gone off and completely spoiled at room temperature by the next week (i.e. Day-8).

Figure 4.5 depicts the dielectric permittivity vs. frequency graph for third category of milk product under test - the whole milk sample. Same depiction is made for whole milk where numbers on the right show temperature in °C and D1 through D8 indicate Day1 through to Day8, respectively. Likewise other two milk types, the trend looks similar and Day-8 is an obvious standing out measurement due to spoilage at its peak of all eight days.

In all the cases, Day-8 was on the following Monday (Day-8) after starting the tests on the previous Monday (Day-1) and clots were formed with separation of solids from water.

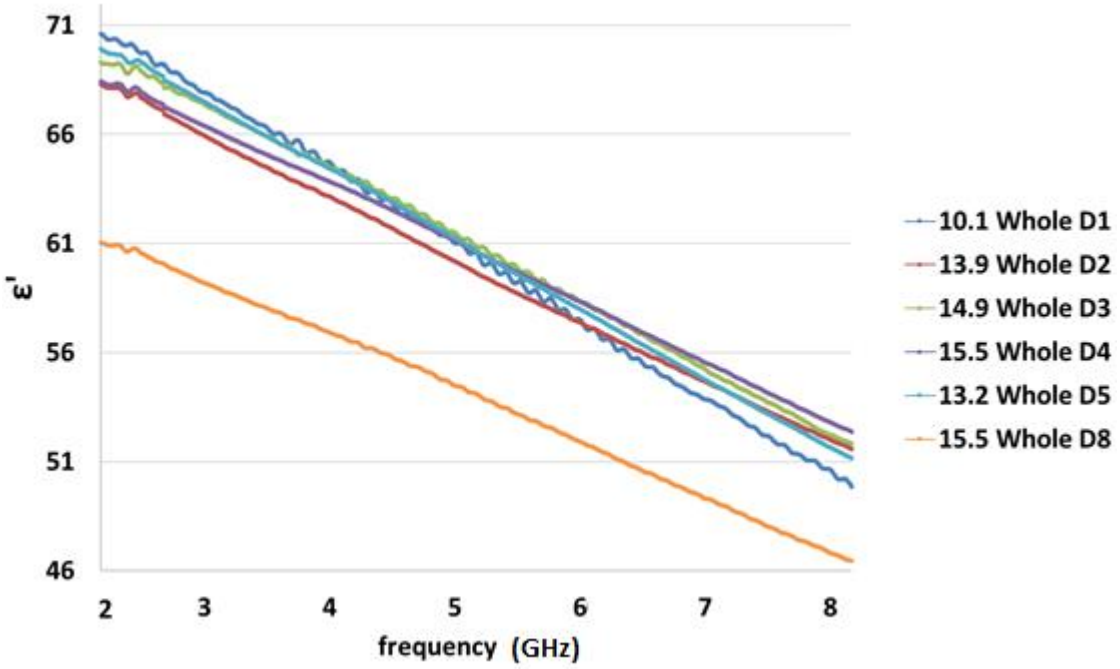


Figure 4.5 Dielectric Permittivity vs. Frequency values for Whole milk samples Day-1 to Day-8

4.3 Resistance and Capacitance Analysis of Milk

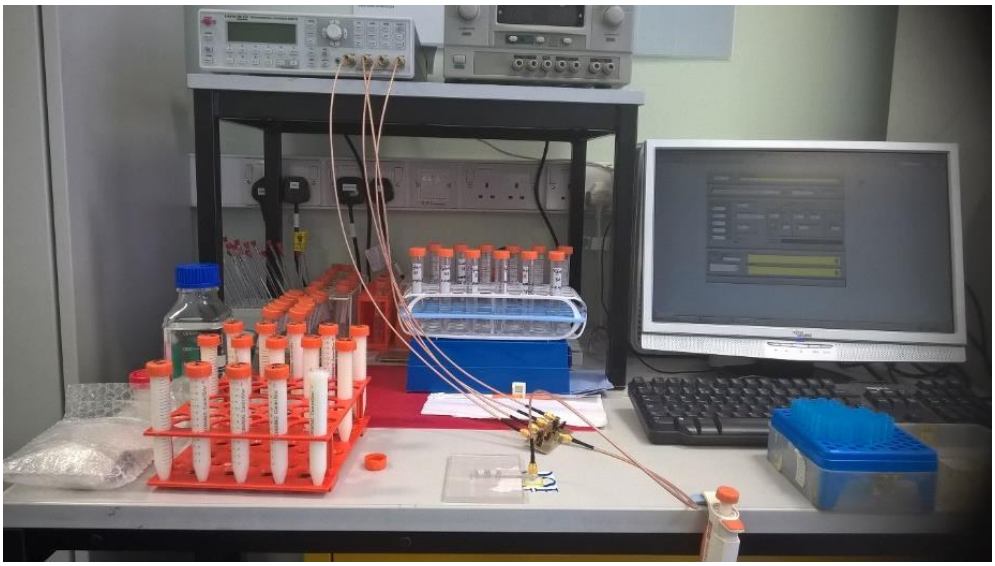


Figure 4.6 HAMEG instruments LCR bridge circuit setup

Figure 4.6 and Figure 4.7 show the experimental set-up for Resistance and Capacitance analysis of three types of milk samples using HAMEG instruments LCR Bridge analysis circuit.

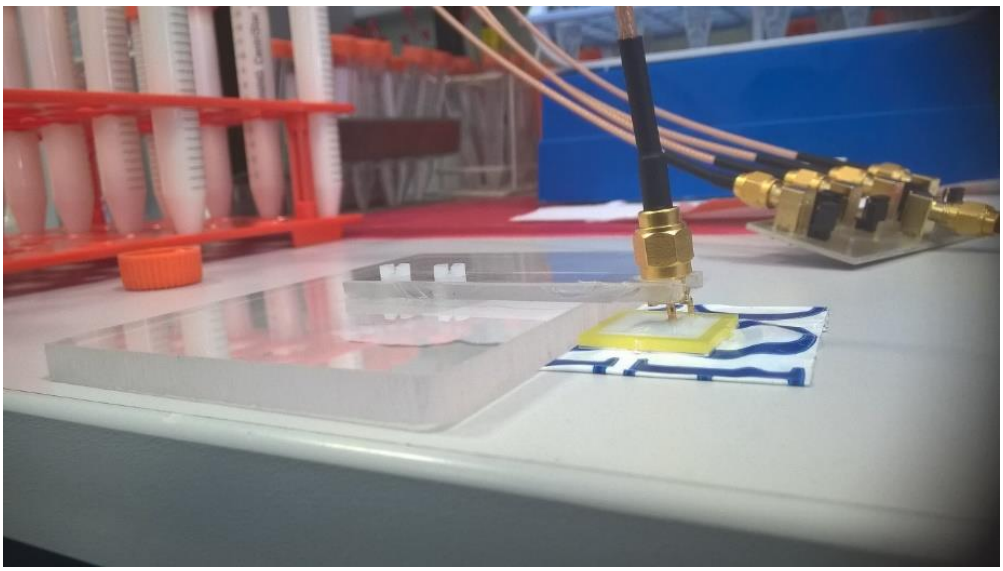


Figure 4.7 The probe inserted in the milk sample for LCR Analysis

Figure 4.8, Figure 4.9 and Figure 4.10 show Resistance values for skimmed milk, semi-skimmed milk and whole milk for 1-week period (5 days) in R (Ω) vs. f (Hz) plots for days 1 to 5 respectively.

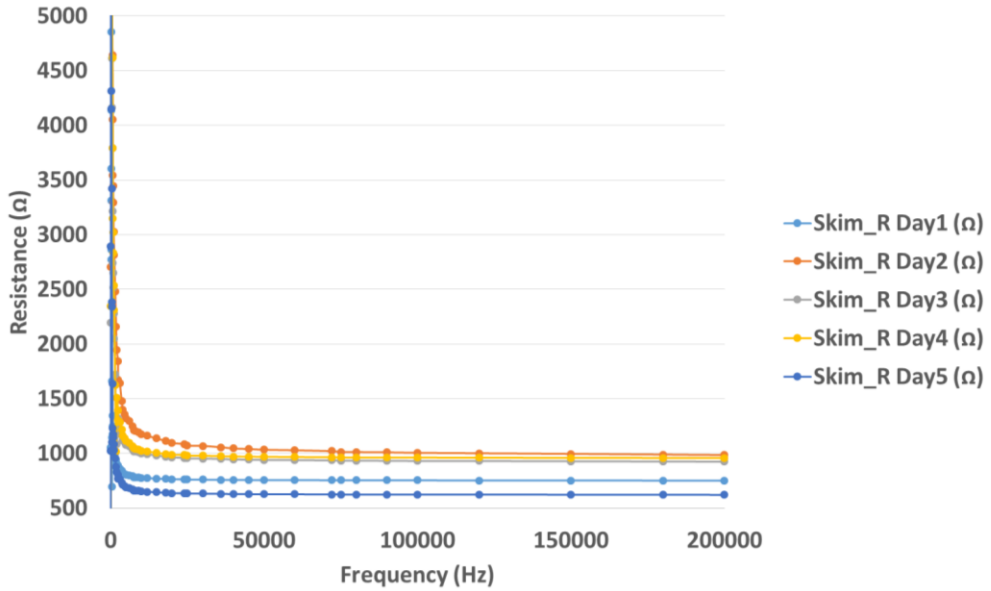


Figure 4.8 Resistance vs. Frequency values for Skimmed Milk (Day-1 to 5)

Observations made on the measurements results achieved shows that in all three milk types, the values of corresponding electrical resistance of milk sample (size, 500µl) under test decreases with rise in the frequencies.

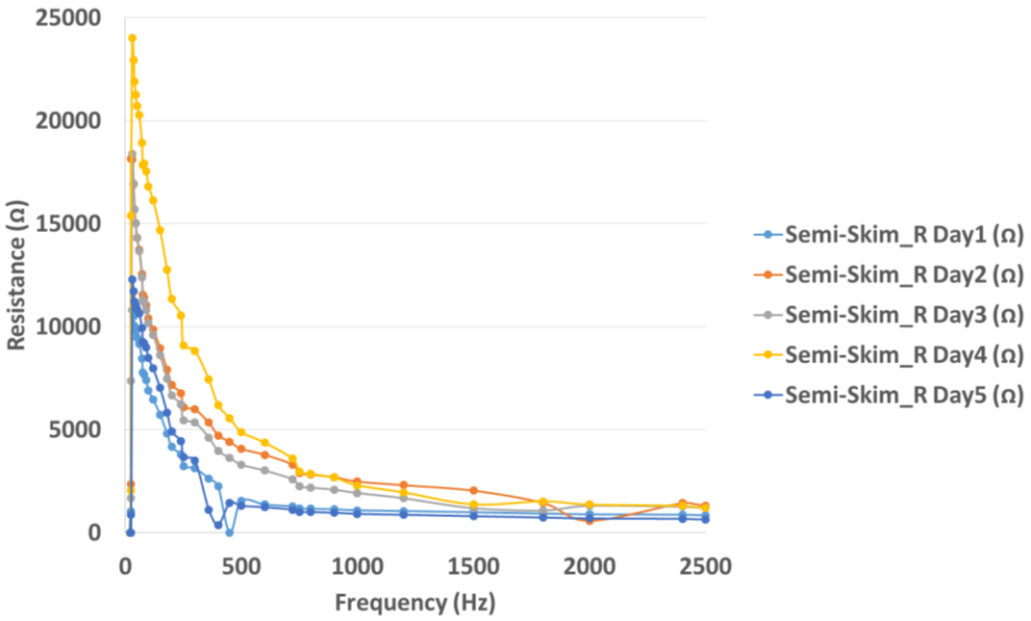


Figure 4.9 Resistance vs. Frequency Values for Semi-skimmed Milk (Day-1 to 5)

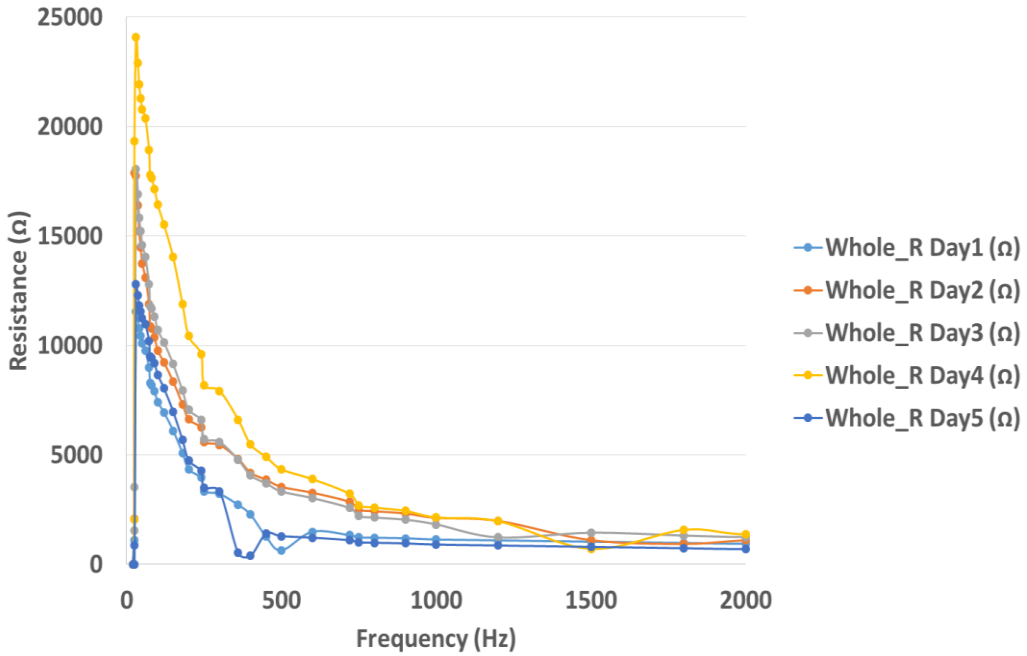


Figure 4.10 Resistance vs. Frequency values for Whole Milk (Day-1 to 5)

Figure 4.11 and Figure 4.12 on the other hand show direct comparison among three milk types with respect to their Resistance and Capacitance values vs. the frequency, respectively, on the Day-1 of observation period of the test week.

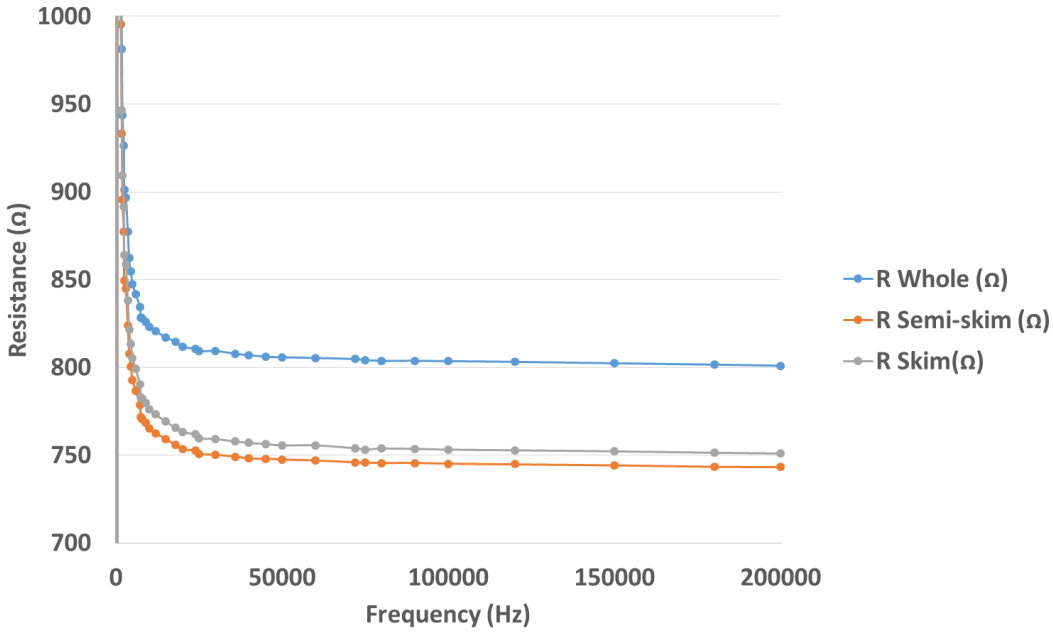


Figure 4.11 Resistance vs. Frequency for all three milk types (Day-1)

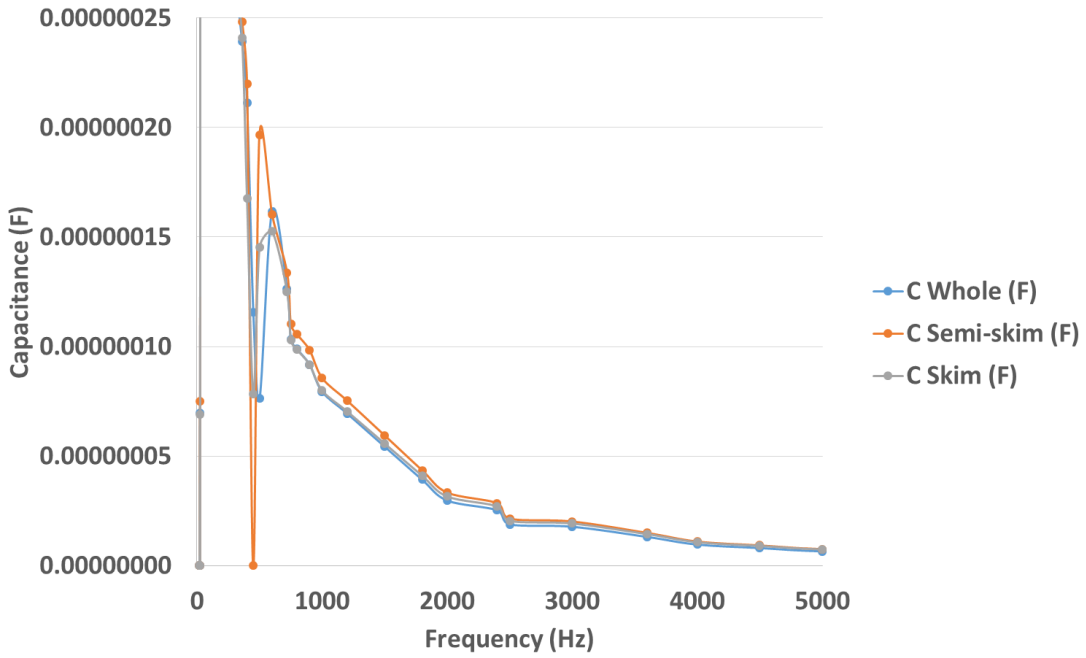


Figure 4.12 Capacitance vs. Frequency Values for all three Milk Types (Day-1)

The resistance values hold critical information more than that of the capacitance values of milk. As can be seen, there is a clear separation of R-values with the different milk types as well as the number of days passed.

4.4 Microwave Sensor Design

4.4.1 Microwave Resonator Cavity (Sensor A)

There are several parameters, which decide the accuracy of the results in dielectric materials measurements. Q-factor is one of the most important factors for estimating the quality of a resonator sensor, the higher the Q-factor means the higher the accuracy and narrower the bandwidth. The Q-factor depends upon many conditions such as the metal used for constructing the cavity resonator, the filled material under examination (MUT), the coupling device (connectors and impedance matching) and the transverse modes (Li and Jiang, 2010).

The resonance frequencies, in microwave cavity sensor, for TE mode and TM mode equations for microwave cavity are given by Eq. (3.8) and Eq. (3.9), respectively, (Wangler, Wangler and Wiley InterScience (Online service), 2008):

$$f_{mnp} = \frac{c}{2\pi\sqrt{\mu_r \epsilon_r}} \sqrt{\left(\frac{X_{mn}}{R}\right)^2 + \left(\frac{p\pi}{L}\right)^2} \tag{3.8}$$

$$f_{mnp} = \frac{c}{2\pi\sqrt{\mu_r \epsilon_r}} \sqrt{\left(\frac{X'_{mn}}{R}\right)^2 + \left(\frac{p\pi}{L}\right)^2} \tag{3.9}$$

Where, *R* and *L* are radius and length for a cylindrical cavity, respectively. X_{mn} represents nth zero of mth Bessel function and X'_{mn} represents nth zero of derivative of mth Bessel function. μ_r and ε_r are relative permeability and relative permittivity of the medium, respectively.

Figure 4.13 illustrates the HFSS simulation model for the microwave, resonator cavity (sensor A) designed at LJMU (Goh *et al.*, 2011), which can hold a test-tube with sample, to employ for milk quality test.

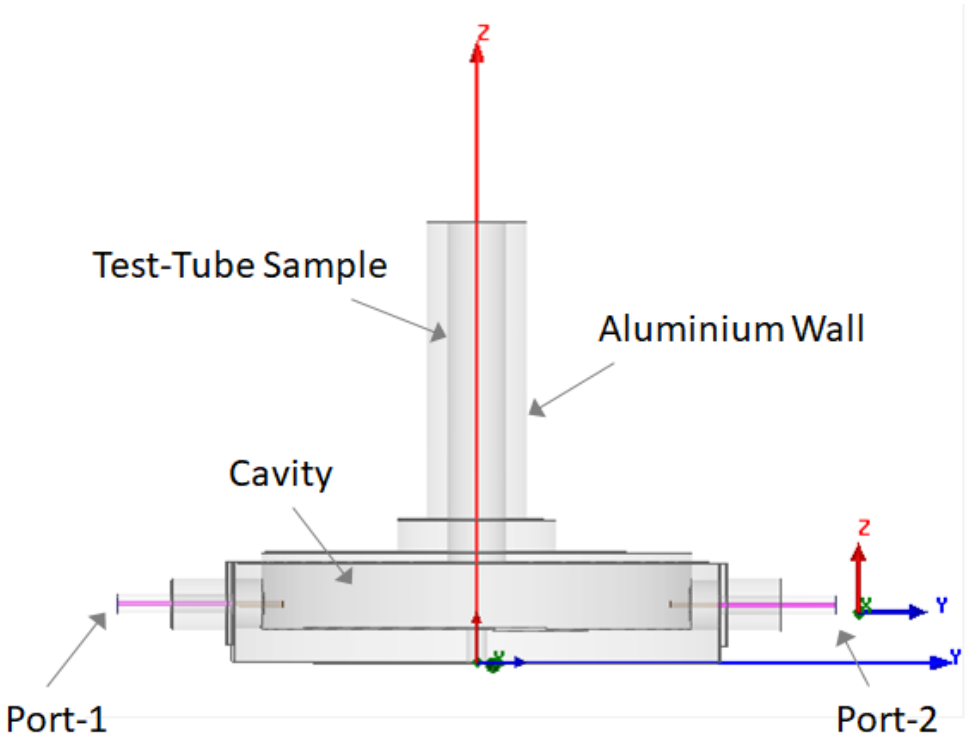


Figure 4.13 HFSS Simulation Model for 2-port Microwave Resonator Cavity Sensor

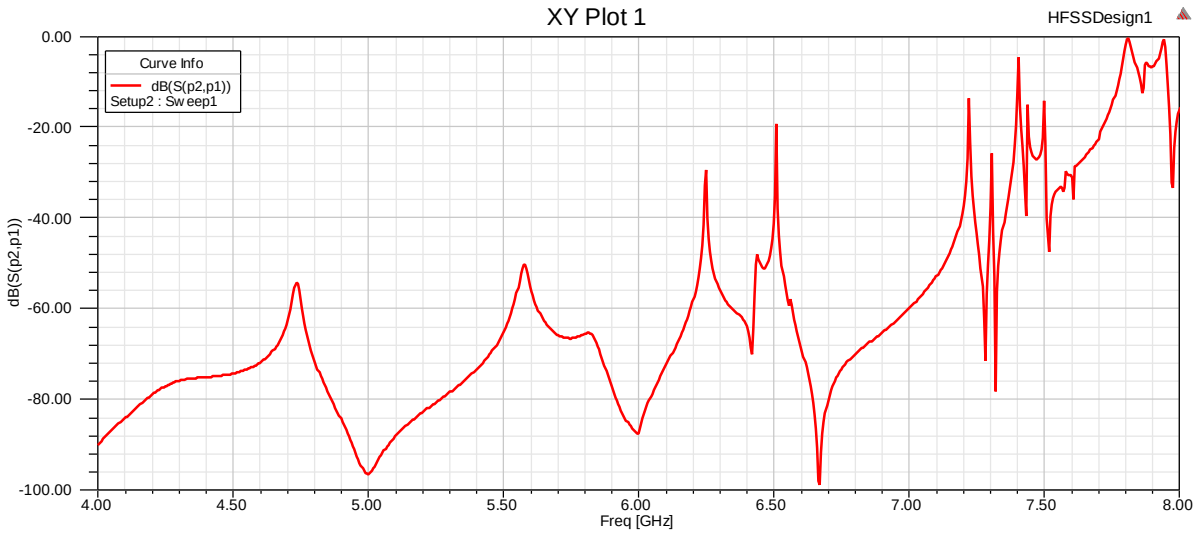


Figure 4.14 HFSS simulation: S_{21} vs. Frequency plot for the microwave cavity sensor

Figure 4.14 demonstrates the simulation graph of S_{21} against frequency between 4 GHz to 8 GHz band, using HFSS, whereas Figure 4.15 illustrates the same graph for the fabricated sensor hardware using the same values of runtime frequencies, which is recorded by a vector network analyser and plotted accordingly. The body of the sensor A is made up of aluminium metal, whereas the radiating probes connected to two ports are manufactured using copper.

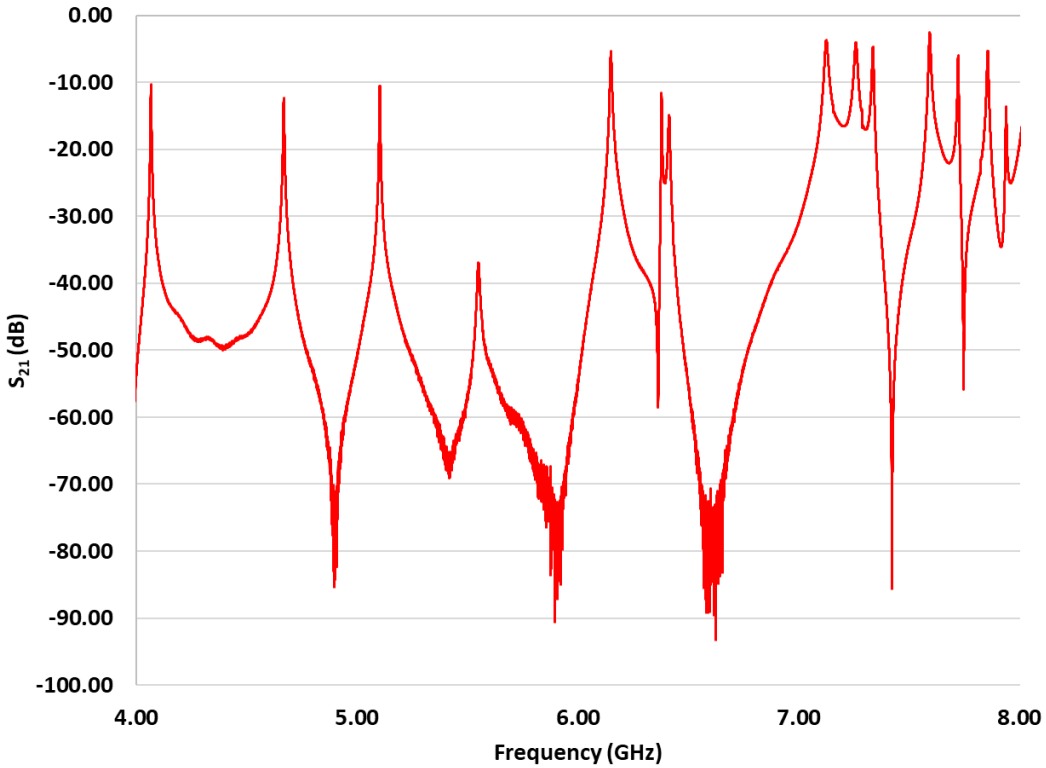


Figure 4.15 S_{21} vs. Frequency plot for the fabricated microwave Cavity Sensor

The variation in the simulated results as compared to the manufactured sensor result appear due to possibility of variations in manufactured dimensions differences to that of simulated sensor A. Furthermore, the change in connector types and/or dimensions could result in change of corresponding results with measurement results in real-world microwave sensor.

4.4.2 Inter-Digitated Electrodes (Sensor B)

Interdigitated Electrodes (IDEs) are mainly designed as filters (Singh, Soni and Nirmal, 2018) or antenna designs (Wahba, Abdalla and Allam, 2016) that are compact in size and light in weight. Particularly due to extraordinary feature of the IDEs; the microstrip-line-excited IDE exhibits band-stop characteristics, essentially, working as a near-field, sensitive, sensor surface probes. Moreover, the IDE probe has are simple in structure, easy to operate, and multifunctional with a low fabrication cost (Li *et al.*, 2018). In recent times, IDEs are also being widely used as sensors to monitor changes in dielectric properties of materials under test (MUT) (Bao *et al.*, 2018) and gas sensors (Alcantara and Andrade, 2016), or even for the surface defect detection in metallic materials (Abdullah *et al.*, 2014).

The IDE sensors possess high enough sensitivity to distinguish the variation within closed regions. The minimum transmission coefficient frequency is dependent on the resonance frequency of the IDE design. Therefore, in an IDE microstrip band-stop filter, the resulting shift in the minimum transmission frequency shows that the IDE filter can be employed for dielectric characteristics test of the MUT, i.e. milk sample in this case. This phenomenon is further highlighted in milk type quality test results using IDE sensors in Chapter 6, using three different milk categories, namely skimmed, semi-skimmed, and whole milk.

The frequency, bandwidth, and time response of the IDE are variables that can be controlled by the number of finger electrode pairs in both the sensor probes on surface, the distance between the generating and receiving sensor probes, the overlap region of the fingers, and the width and spacing of adjacent fingers (Hickernell, 1998). The velocity of these waves depends on the dielectric and mechanical properties of the MUT. The phase shift (Φ) of the sensor signal is given by Eq. (3.10) (Mamishev *et al.*, 2004):

$$\Phi = \omega \frac{L}{v} \tag{3.10}$$

Where v is the wave speed, ω is the frequency of excitation, and L is the length of the transducer, when IDEs are used to build the Surface Acoustic Wave (SAW) device. The main principle of sensing for IDE capacitive sensors is based on the

change of the dielectric constant of the interdigitated surface capacitor. Here, the change of the capacitance occurs when the properties of the dielectric of the material between the plates changes. The capacitance of the IDE sensor is stated in Eq. (3.11) (Mazlan *et al.*, 2017):

$$C_{Sensor} = \eta\varepsilon \frac{lt}{d} \tag{3.11}$$

where η is the number of electrodes, ε is the dielectric permittivity of the sensitive coating film, l is the length of IDEs, t is the thickness of IDEs and d is the distance between the electrodes.

Figure 4.16 depicts the HFSS simulation model of the IDE (sensor B) designed at LJMU (Blakey *et al.*, 2012; Korostynska, Mason and Al-Shamma'A, 2012).

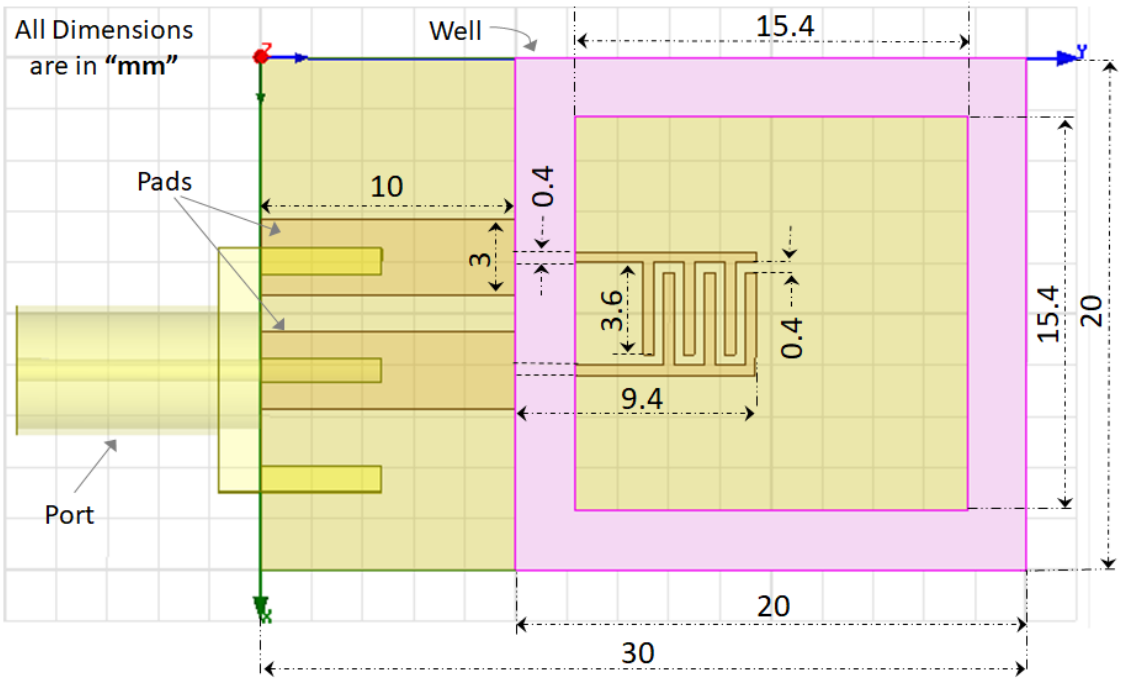


Figure 4.16 HFSS Simulation Model for 1-port Microwave IDE

The sensor B used here carried 3 pairs of electrodes cross-coupled against each other as can be seen from the figure above. The Gold plated electrodes were particularly used to achieve higher conductivity as well as protection for the probes against corrosive liquids under test, when they directly are exposed to them due to being invasive technique of material property testing. Figure 4.17 illustrates the simulated results of the sensor B using HFSS modelling tool.

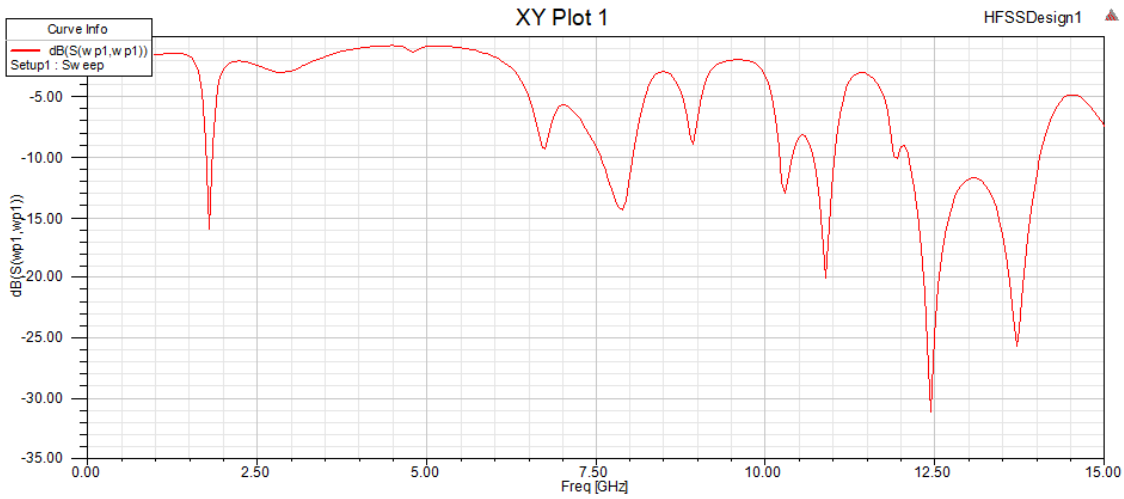


Figure 4.17 HFSS Model Simulation S_{11} vs. Frequency Plot for the IDE Sensor

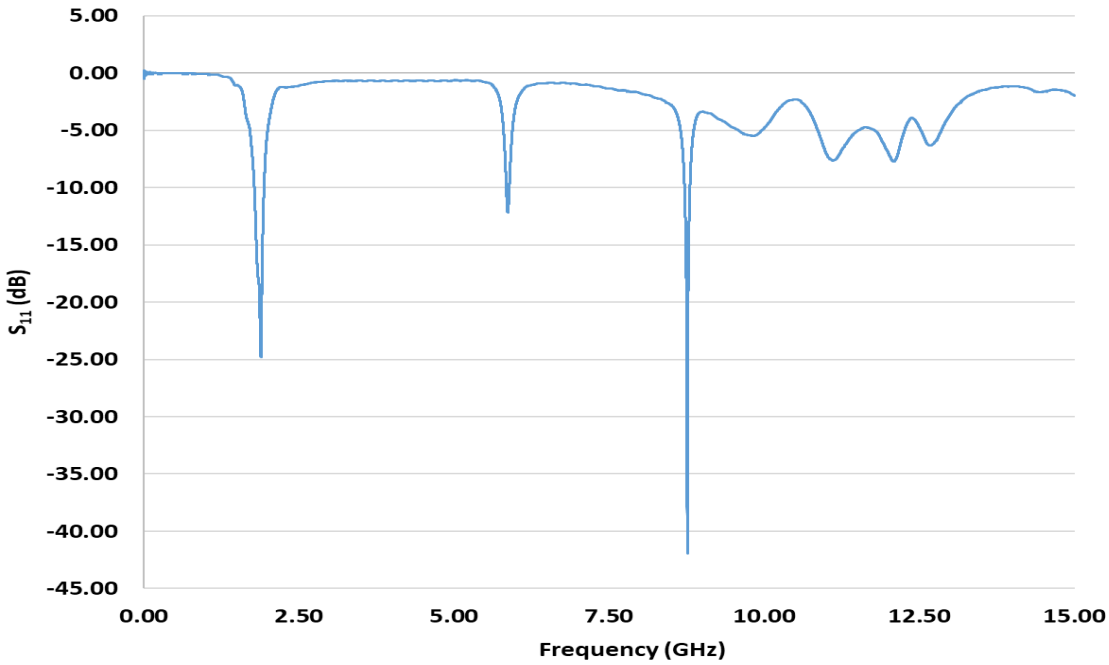


Figure 4.18 S_{11} vs. Frequency Plot for the Fabricated Sensor B

Figure 4.18 illustrates S_{11} vs. Frequencies plot for the fabricated sensor B, with three distinctive resonant frequencies.

4.4.3 Microwave Fluidic Planar Resonator (Sensor C)

These types of planar resonator sensors behave as band-pass filters, which allow a wideband of frequency to pass based on its design constraints. Rising demand for mobile and radio tele-communication has caused a significant lack of frequency resources lower than 1 GHz (Sagawa, Takahashi and Makimoto, 1989). This surge in wireless communication and mobile application paved a way for higher frequency band applications. The cross-coupled filters, such as the ones utilised in the hairpin resonator design, are particularly of interest as they exhibit ripples in both passband and stopband, which has been reported to improve both on frequency selectivity and in band-pass loss (Hong and Lancaster, 1998).

In addition, the microwave spectroscopy allows for a non-invasive and non-destructive way of sensing the material under test, meaning that the samples need not be destroyed or spoiled with adding reagents from their normal use or consumption. This sensing technology is relatively cheap compared with other expensive techniques discussed in the previous chapter and planar sensors such as this one can cost well under £400 overall.

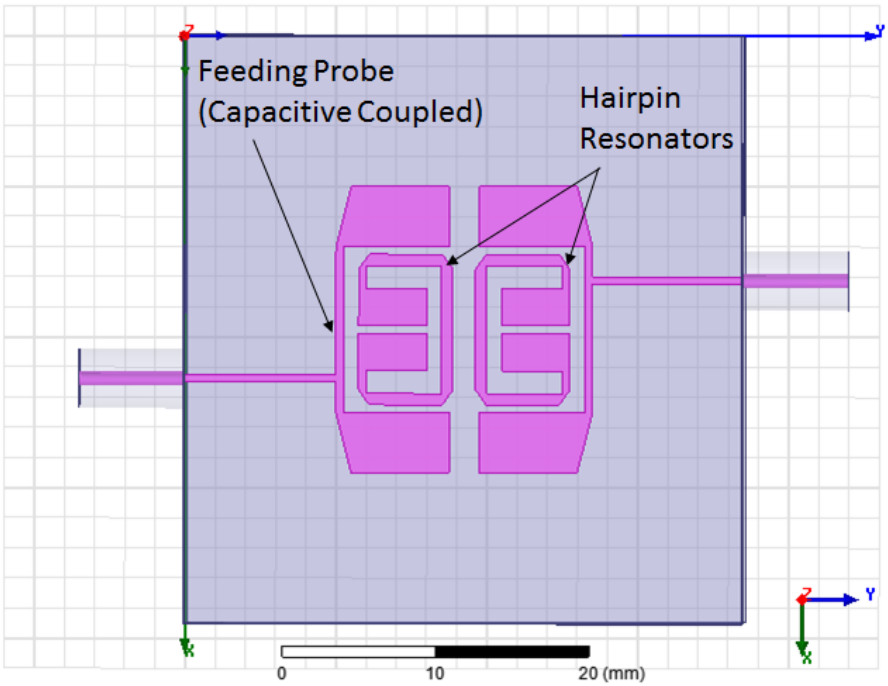


Figure 4.19 HFSS Simulation of Fluidic Sensor (Hairpin Resonator)

Figure 4.19 shows the HFSS simulation model screenshot of modified hairpin resonator (sensor C,). Researchers Liu *et al.* (2018) have used similar hairpin resonators for microfluidic sensing of NaCl concentrations with Rogers as its dielectric substrate. In this research, the substrate used for fabrication of the resonator sensor was made up of standard Reinforced Fibre (FR4-epoxy) material, which is easily available and relatively cheaper compared to other dielectric material types.

The primary advantage of these sensors is that they have universal applications unlike dedicated sensors (e.g. not specific to bacteria/lipid/protein type but instead aim to detect overall spoilage). These sensors also have better accuracy and omit the tedious stage of reagents preparation, saving time and resources.

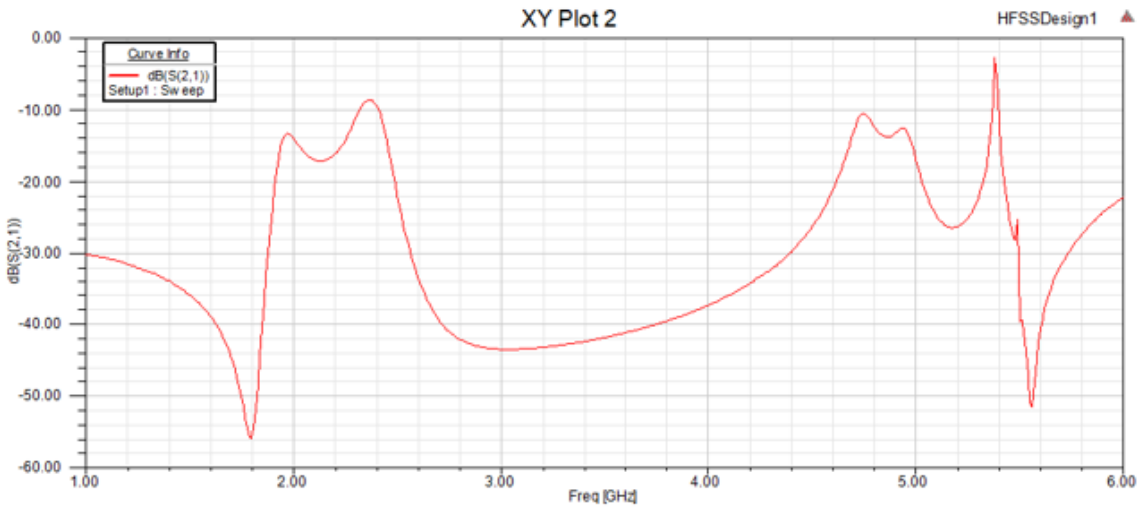


Figure 4.20 Simulation Results of Hairpin Resonator Sensor

Figure 4.20 gives simulation results of the fluidic sensor for S_{21} vs. frequency plot. and Figure 4.21 shows the same graph corresponding to actual fabricated sensor. The variations in simulation results and actual fabricated sensor is due to several factors like, type of ports used, differences between actual materials used for fabrication vs. the materials used in simulation package etc.

These types of sensors behave mainly as large band-pass filters allowing broad range of frequencies in sensing and detection tests.

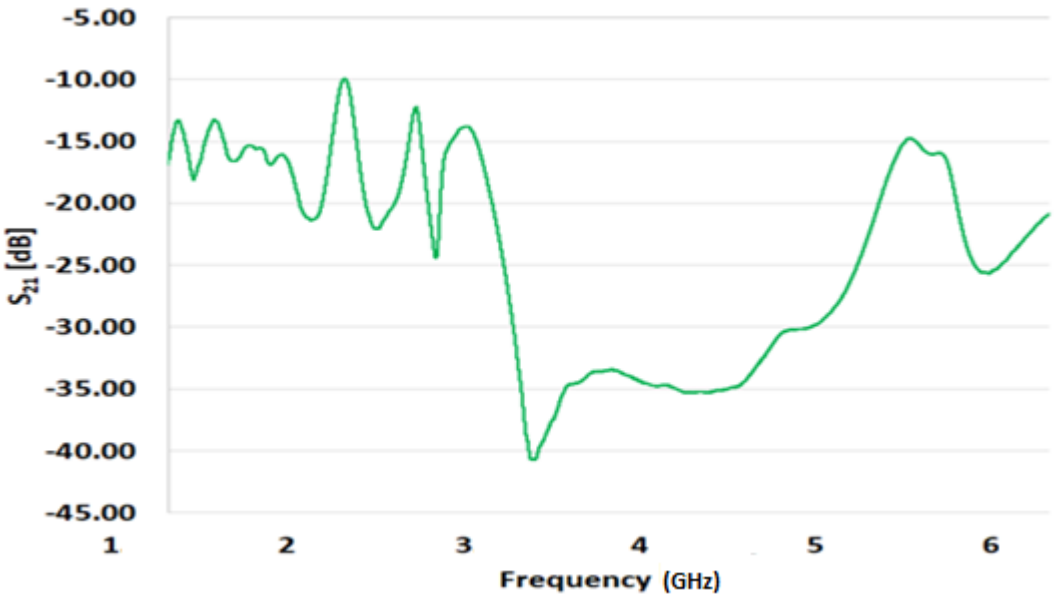


Figure 4.21 S_{21} vs. Frequency plot for the fabricated planar Hairpin Resonator Sensor

The Printed Circuit Board (PCB) layout was prepared using EAGLE software from Autodesk. The patch sensor was printed on the planar resonator surface, with copper as conducting patch material and ground plane, whereas, reinforced fibre epoxy (FR4) was used as the dielectric.

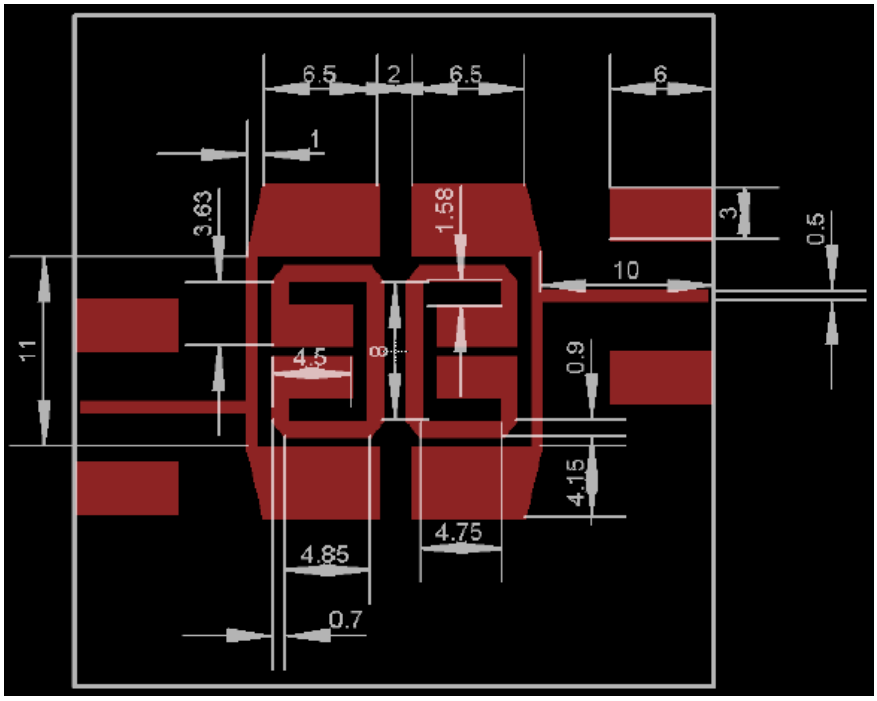


Figure 4.22 The Screen Printing EAGLE Board Layout of Hairpin Resonator (all dimensions in mm)

The designing parameters in terms of Autodesk EAGLE board dimensions of the fabricated resonator sensor are depicted in Figure 4.22 as screenshot of the board design. Figure 4.23 shows the sensor C after fabrication with 2-ports planar structure and applicable to fluidic sensing systems where samples can be carried through a tube.

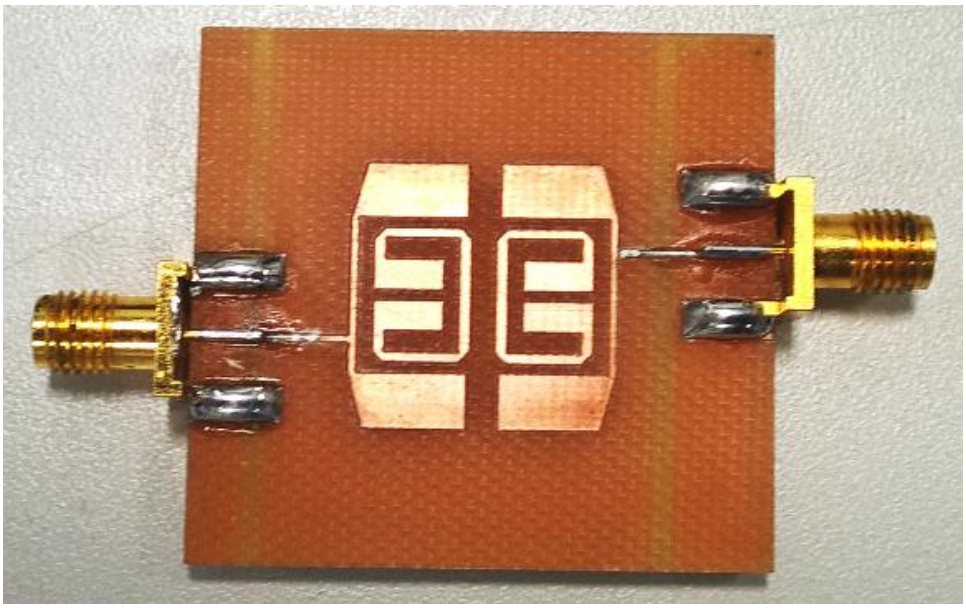


Figure 4.23 The Fabricated Planar Hairpin Resonator Fluidic Sensor

4.5 Methodology involving Microwave Sensors

The three major categories of milk, commercially sold, in the market whole, semi-skimmed and skimmed milk were tested. The measurement set-up uses a Rhode & Schwarz Vector Network Analyser (VNA), which was connected with the microwave sensors to record the spectral signatures in terms of scattering parameters.

For all these three categories, the spoilage process of milk was examined with the help of a microwave sensor A and sensor B, as microwave sensing devices, to establish generic characteristics of the system. Then a fluidic sensor (sensor C) design was developed and optimised to examine milk when the sample is carried through the planar resonator via a polyethylene tube using a peristaltic pump (see Figure 4.24).

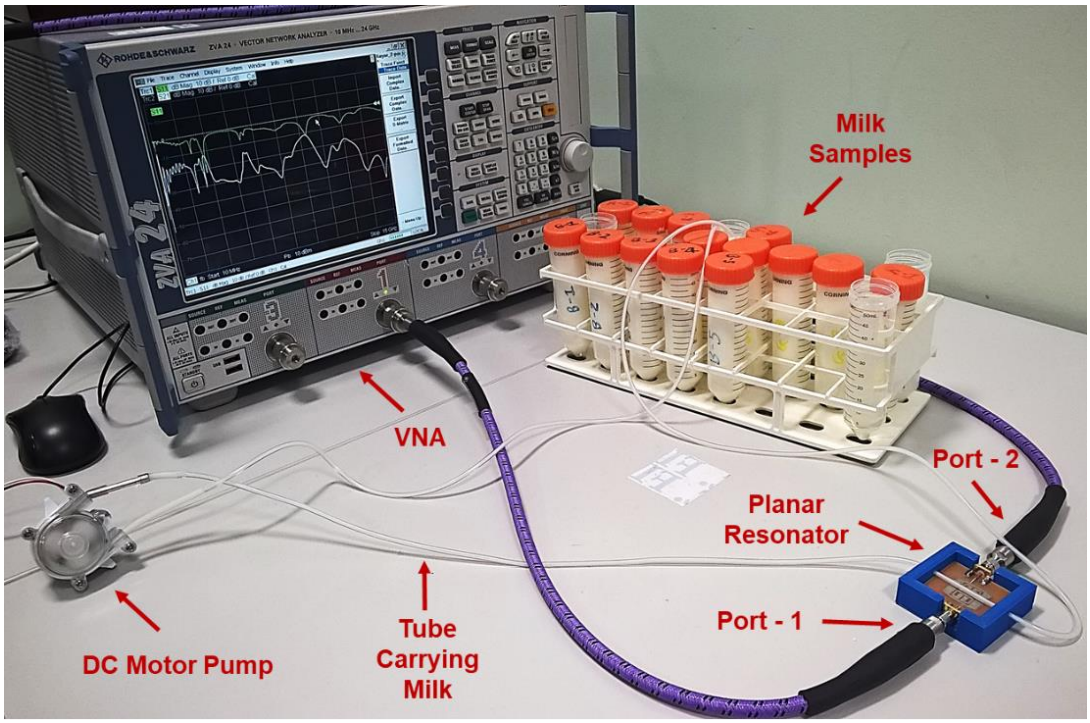


Figure 4.24 Experimental set-up for milk quality analysis using fluidic sensor

This comprised the detection and analysis of spectral signatures for S_{11} and S_{21} parameters, connectively known as scattering parameters, which are the measures of reflected back energy (power) towards the input Port-1 and transferred energy (power) towards the input Port-2, respectively, in the EM wave sensor assembly.

4.6 Summary

Here, the dielectric property of each milk type was achieved using the single coaxial probe analysis method and the three milk types were clearly distinguished from one another. Also, it was proven that the test results complimented the conclusions derived by other researchers in the past by showcasing that the dielectric parametric values were decreasing with respect to frequency values and with regards to the fat content.

RC analysis of each milk type was also carried out and differences studied and explained with respect to Resistance and Capacitance values of each milk type under consideration, i.e. skimmed milk, semi-skimmed milk and whole milk.

The three different sensor types were simulated and studied using simulation as well as fabricated results. Chapter 5 explains the use of a novel methodology to detect the spoilage in milk, over a period of time, using EM wave sensing of milk.

CHAPTER 5

DETECTING SPOILAGE IN MILK SAMPLES

5.1 Background

Milk is a perishable product and spoils quicker than other food items if not stored in favourable conditions such as ideal temperatures. All milk lasts 5-7 days past its printed date if refrigerated, once opened (Eat By Date, 2018). Its nature of having lower acidity and higher nutrients makes it a very suitable breeding platform for bacterial microorganisms known as pathogens, which are responsible for food poisoning. Bacteria contaminate the milk and milk processing is mainly done to destroy them, which enables milk preservation. The milk preservation is achieved by various procedures of heating, cooling, fermentation, removal of water, and concentration or separation of various components from milk to produce foods like cheese and butter (Fellows and Hampton, 1992).

Spoilage detection techniques play an important role in improving the milk preservation methods. Milk spoilage could be defined in several ways with its measure being different each time based on the context it is discussed in, e.g., for a common buyer, the simplest definition, of a quality product would be, a product that smells as well as tastes good (Murphy, 2009). Adulteration check has also been one of the sustained interests in the milk quality control process, considering the ambiguity of “Use by Date” and its relevance to the actual spoilage of the milk as this vagueness can trouble both the consumers and the manufacturers. Buyers mostly avoid purchasing products that are close to their dates of expiry. This eventually imposes a negative financial impact on the entire dairy industry (Lu *et al.*, 2013). Hence, there is an ongoing need for an accurate spoilage detection technique in milk as much as there is a growing demand for prevention of the wastage of milk and the illnesses occurring due to the consumption of deteriorated milk.

Several criteria for the spoilage of milk were observed and discussed including bacterial growth within the skimmed and whole milk types (Deeth *et al.*, 2002) and it was observed that the bacterial growth patterns in the skimmed milk did not significantly differ from those of the whole milk.

Additionally, it was also confirmed by their study that the spoilage bacteria in milk grow at similar rates, in skimmed milk as well as whole milk, but both types have different metabolic behaviours. This complements the results achieved in this work as explained in this chapter along with that of the other researchers who observed similar rates of growth in skimmed and whole milk types (Janzen, Bishop and Bodine, 1982)(Brown, Ranjith and Prentice, 1984)(Chandler, Ng and Hull, 1990).

These findings infer a common conclusion that the different shelf lives of skimmed and whole milk types are not characterised by different bacterial growth rates within them.

5.2 Methodology and Measurements

This section, explains the procedures adopted to carry out the milk quality sensing, in terms of spoilage detection, using existing simple resonator cavity (sensor A) and Inter-digitated Electrodes sensor (sensor B) designs, in order to develop an idea of responses for each of them.

5.2.1 Sample Preparation

Table 5.1 The nutritional components of the three milk types under test

Typical Values (per 100 ml)	**Milk Categories**		
	Skimmed milk	**Semi-skimmed milk**	**Whole milk**
Energy (kJ)	147 (35 kcal)	209 (50 kcal)	268 (64 kcal)
Fat (g)	0.1	1.8	3.6
Saturates (g)	<0.1	1.1	2.3
Fibre (g)	0.0	0.0	0.0
Protein (g)	3.4	3.6	3.2
Carbohydrates (g)	5.0	4.8	4.7
Sugars (g)	5.0	4.8	4.7
Calcium (mg)	124.0	124.0	120.0

Table 5.1 lists the nutritional content of the three milk types used for making the test samples. These three milk types were purchased from the market, at Tesco superstore. Three milk-types; skimmed, semi-skimmed and whole milk were used

to make five samples from each using 15 ml size polypropylene plastic test-tubes (Joshi *et al.*, 2015). The sample making was done at room temperature and the storage was governed by temperature controlled climate with minimum variations as shown in Figure 5.1 and Table 5.2.

For the better reliability of the captured spectral data from EM wave sensor, 5 repetitions for each of the 5 samples for all 3 categories of milk, were made, giving eventually 75 total iterations to be mathematically analysed. The test-tubes were labelled as R-1, R-2, ... , R-5 for skimmed milk, G-1, G-2, … , G-5 for semi-skimmed milk and B-1, B-2, ... , B-5 for whole-milk as they are colour-coded as Red-Top, Green-Top and Blue-Top, respectively, in the commercial market (Figure 5.1). One extra test-tube was dedicated to detect any variations with a digital thermometer probe inside to ensure steady temperature conditions in the lab. Test tubes with deionised water and air were also tested to see sensor response.

Figure 5.1 Sample preparation: milk spoilage detection using EM wave sensors

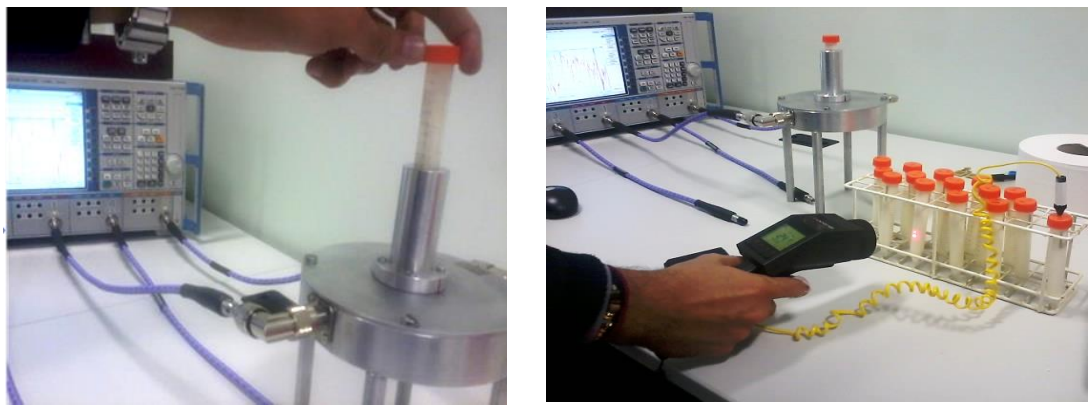
5.2.2 Sensor A: Measurement conditions

For the spoilage detection tests using the microwave resonator cavity (sensor A), a slot that can hold the 15 ml test-tube carrying milk samples, was provided as shown in Figure 5.2. This 2-port resonator sensor A was calibrated with the R&S®ZVA vector network analyser to enable accurate measurements of data in terms of scattering parameters, to demonstrate the effectiveness of the proposed method.

For the measurements a 2-port sensor A was used along with network analyser, as shown in Figure 5.2a. A laser beam thermometer was also used with the digital probe thermometer to ensure stable temperature conditions before, during and after the measurements (see Figure 5.2b,). Through Open Short Match (TOSM), 2-port calibration was used. Table 5.2 depicts the test measurement conditions with sample storage specifications.

Table 5.2 Measurement specifications for resonator cavity (sensor A): Spoilage Detection

Specifications	**Values**
No. of Measurements	3 Types × 5 Samples × 5 Repetitions = 75 Iterations
Sample Size	15 ml (Polypropylene Test Tubes)
Supervision Period	1 week
Temperature Variations	22 °C ± 2 °C
Resonator cavity specifications	Diameter Φ = 130 mm (distance between ports) Height h = 20 mm (height of cavity)
Frequency Sweep	0.01 GHz – 15 GHz (10 KHz Measurement Bandwidth)
Channel Specifications	TOSM calibration / 0 dBm Base Power



(a) (b)

Figure 5.2 Spoilage detection using EM wave cavity sensor

(a) Milk sample under test, (b) Temperature controlled measurement room

Although the sensors are designed at a certain frequency, a wide range of frequency sweep from 10 MHz – 15 GHz was applied in order to identify the frequency region of interest giving best results. This also allowed more spectral data to be captured for comparison before further data analysis within the ultra-wide band of frequency, ultimately giving better results. The distinguishing frequencies were identified for the detection of spoilage for all three types of milk, and its comparison with the previous day's measurements to the following days over a one-week period.

The same procedure was carried out for all milk types, to enable their classification based on composition when they are fresh as explained in Chapter 6 as well as in the case of determining adulteration as investigated in Chapter 7. S_{11} and S_{21} both scattering parameters were plotted, with 0dBm channel base power.

5.2.3 Sensor B: Measurement conditions

For these 1-port microwave devices, the only scattering parameter under consideration is S_{11}, which is recorded with the help of a network analyser. Here also the climate control was maintained at room temperature. 1-port calibration was made to calibrate the sensor with the channel.

(a) (b)

Figure 5.3 Spoilage detection of milk: (a) Measurement setup, (b) Sensor B

Table 5.3 Measurement specifications for sensor B: Spoilage Detection

Specifications	Values
No. of Measurements	3 Types × 5 Samples × 5 Repetitions = 75 Iterations
Sample Size	500 µl (using pipette; held into well)
Supervision Period	5 days
Temperature Variations	21 °C ± 2 °C
Sensor B Specifications	Height of Substrate h_1 = 1.57 mm Height of Substrate h_2 = 1.57 mm (Standard FR4 epoxy)
Materials Used	Electrodes and Ground Plane – Gold plating Dielectric Substrate – FR4 epoxy
Frequency Sweep	0.01 GHz – 15 GHz (10 KHz Measurement Bandwidth)
Channel Specifications	1-port calibration / 10 dBm Base Power

Table 5.3 lists the test measurement specifications for sensor B employed. The electrodes were given gold plating for protection against corrosion due to being in direct contacts with fluids. The well boundaries were designed to hold the liquid milk sample of up to 0.5 ml volume. For this case also, the samples were stored under the same room temperature ensuring minimum variations for a week's period. Each following day, after the first starting day, the samples were agitated (shaken) manually and also using a vertex vibrator for approximately one minute to mix up any separated milk clots comprising proteins and other nutritional constituents from the other water soluble contents, due to increasing bacteria adulteration or spoilage, as shown in Figure 5.4.

It is important to note that the same approach was not followed, for the sensor A, as the sample size was 15 ml test-tube itself being inserted into the resonator cavity, rather than 0.5 ml of sample taken using pipette (see Figure 5.2a). Whereas in the case of sensor B, if the bacteria adulterated, spoiled sample (over the observation period) is not mixed then the measured value could be as a result of separated water soluble content only, which is being able to

make its way to the small opening of the pipette tip. The formed clots would not be able to penetrate through the same.

Figure 5.4 Milk samples being agitated with vertex mixer

In addition, the observation period was kept to 5 days in these tests in place of 7 days due to even the vertex mixer and manual shaking not being able to fully dissolve the separated milk solids with liquid content from the fifth day onwards. Similar approach was taken for sensor type C later on as for sensors A and B.

5.3 Results and Discussion

5.3.1 Using Sensor A

As shown in Figure 5.5 for skimmed milk, there is a clearly visible distinction between the curves for S_{21} plot against frequency values for Day-1 and Day-7. The graph shows the aggregate values of the average of all five repetitions for each of the five milk samples for a given category.

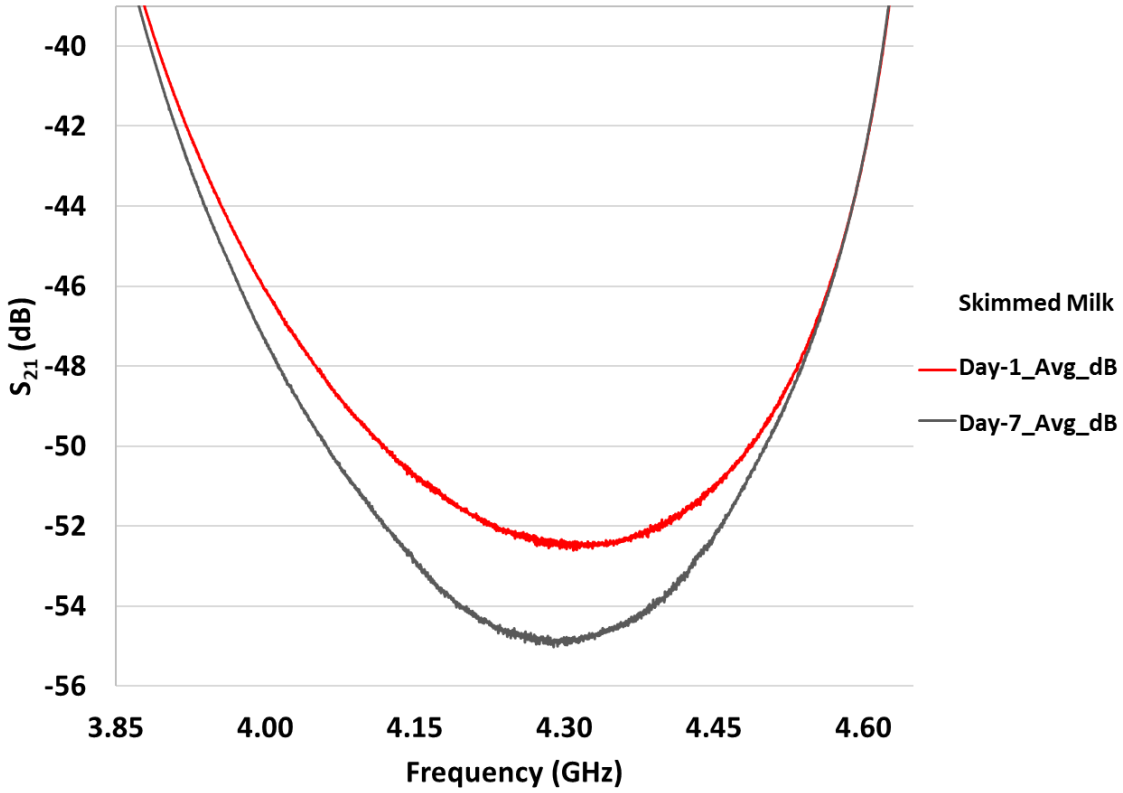


Figure 5.5 S_{21} for Day-1(Red) and Day-7(Grey) Skimmed milk using Cavity Sensor

Similarly, Figure 5.6 and Figure 5.7 show the graphs for semi-skimmed and skimmed milk, respectively.

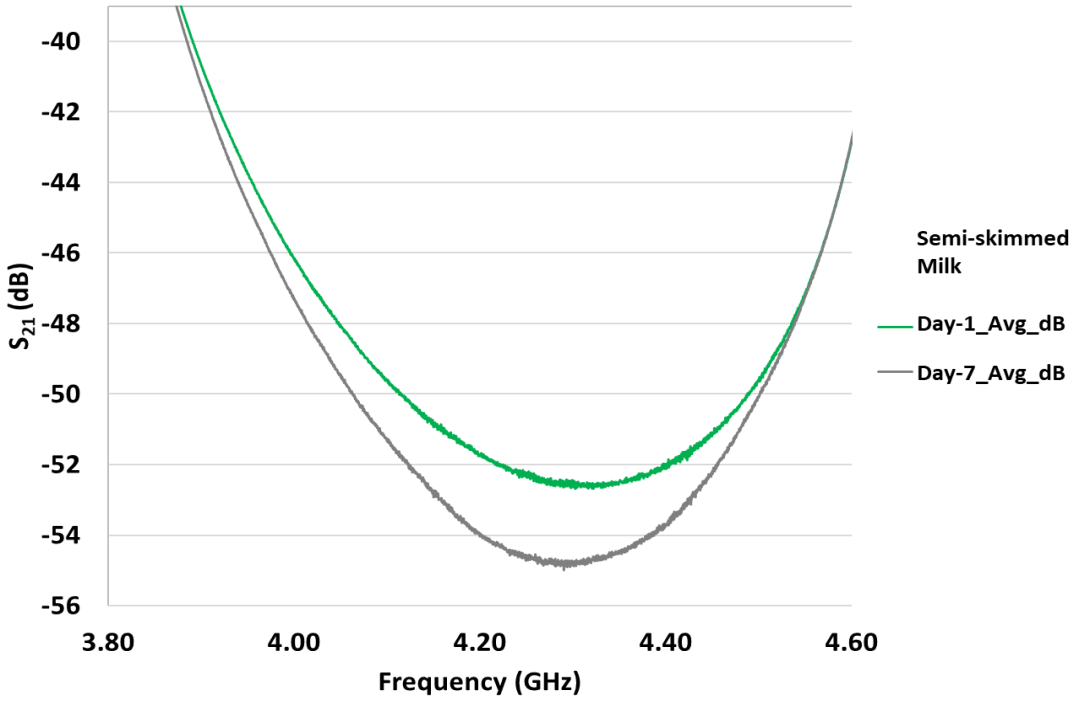


Figure 5.6 S_{21} for Day-1(Green) and Day-7(Grey) Semi-skimmed milk using Cavity Sensor

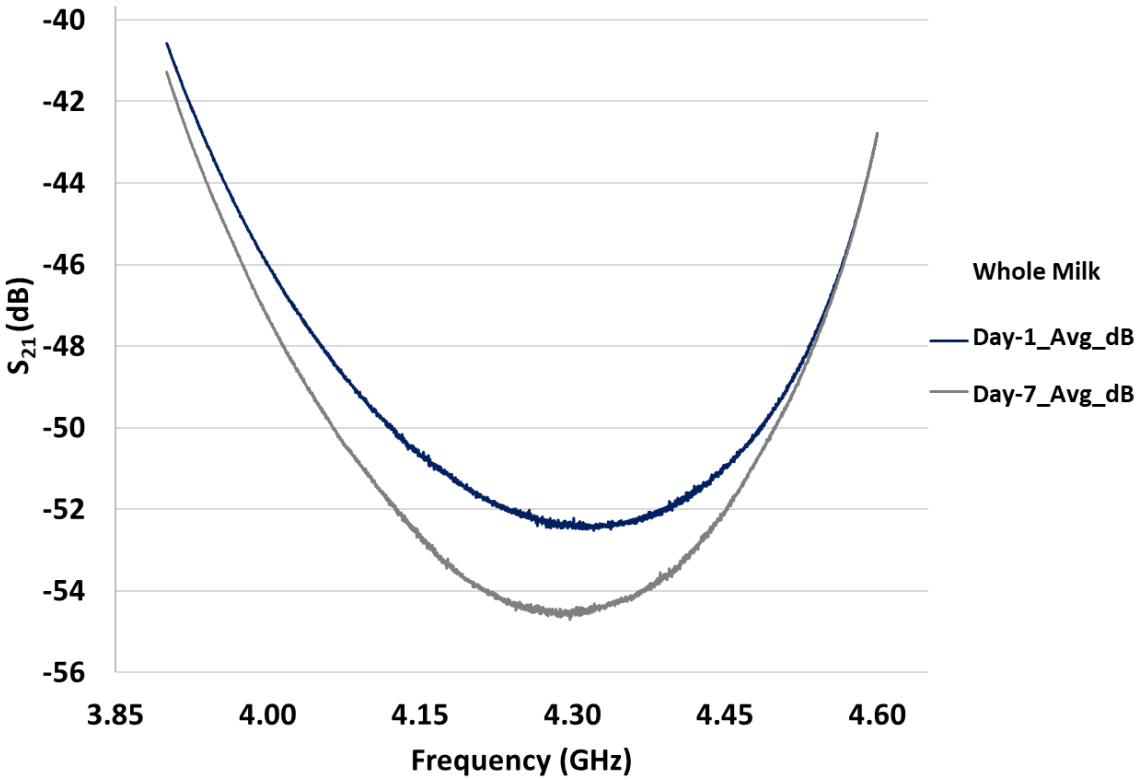


Figure 5.7 S_{21} for Day-1(Blue) and Day-7(Grey) whole milk using cavity sensor

These plots are identical with minor shifts for all three given types of milk and give effective results to distinguish between the aged and fresh milk samples. All milk types gave the response centred at around 4.3 GHz frequency, as can be viewed from the three figures.

5.3.2 Using Sensor B

Using the sensor B for spoilage detection gave a different response, for the same frequency band, the spoilage was seen in terms of frequency shifts rather than the amplitude shifts.

Figure 5.8 shows graph of reflection coefficient (S_{11}) in dB vs. frequency in Hertz. This sensor shows a characteristics frequency shift as the milk type changes from, fresh to spoiled and due to the fact that spoiled milk has water separation from milk solids.

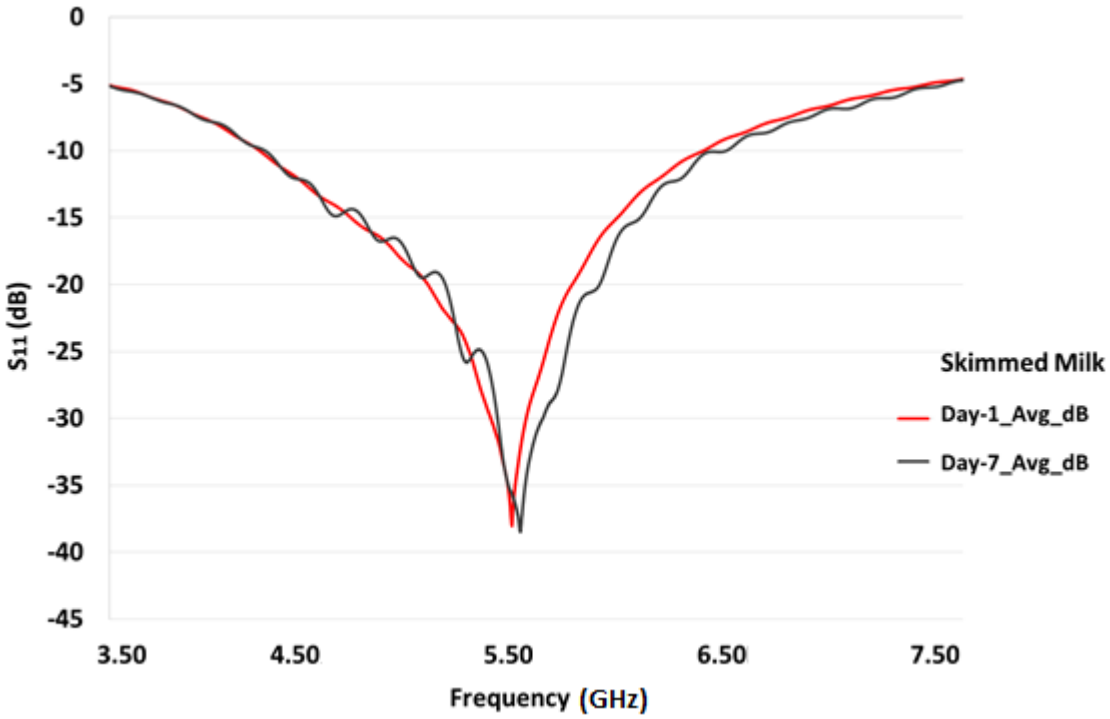


Figure 5.8 S_{11} for Day-1(Red) and Day-7(Grey) skimmed milk using IDE sensor

Similarly, Figure 5.9 and Figure 5.10 show graphs of reflected power (S_{11}) vs. frequency for semi-skimmed milk and whole milk using sensor B, respectively.

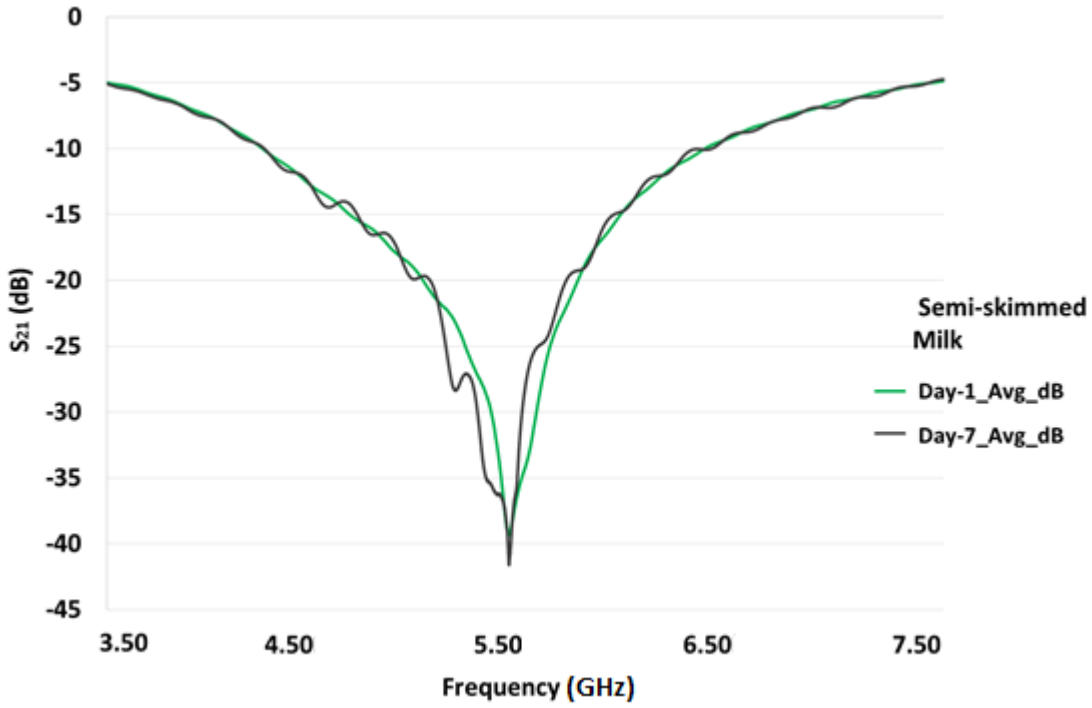


Figure 5.9 S_{11} for Day-1(Green) and Day-7(Grey) semi-skimmed milk using Sensor B

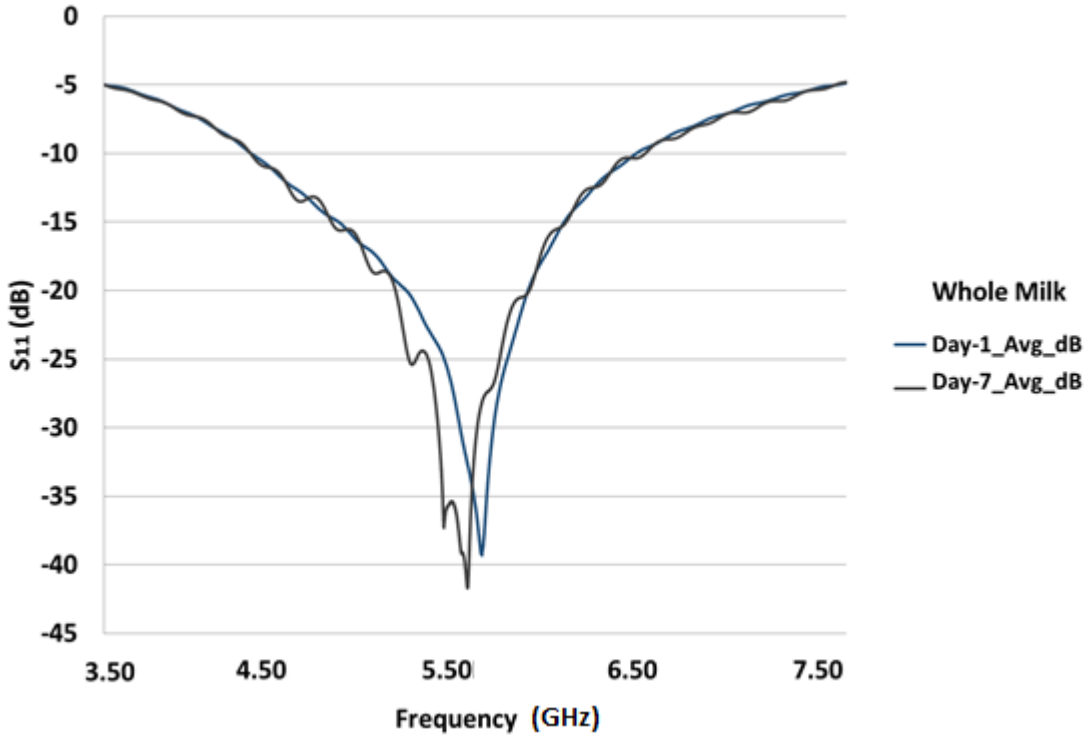


Figure 5.10 S_{11} for Day-1(Blue) and Day-7(Grey) whole milk using IDE sensor

In addition, with the increasing fat content the plots for the Day-7 spoiled milk can be seen shifting towards the left of the centre frequency of about 5.5 GHz, as we go from skimmed milk to whole milk, i.e. with increasing fat content. The visible ripples in the Day-7spectra are present due to the separation of solids from water liquids (clot formation) within the milk samples, despite the mixing stage added in the lab procedure.

The sensor A is acting to detect bacteria adulteration and spoilage in terms of amplitude shifts, whereas the sensor B does the detection by showing frequency shifts, in each of the three categories of milk under test.

A wideband frequency sweep applied to the sensor A helped to identify the values of frequency giving optimum results thereby allowing customization of the final proposed design. Once having located the suitable frequency values, a dedicated microwave sensor acting as a fluidic patch sensor, was designed to operate on the same range of resonant frequencies for an optimized milk quality testing system.

5.3.3 Using Sensor C

Figure 5.11 and Figure 5.12 show Day-1 to Day-5 plots for S_{21} vs. Frequency values for skimmed milk and semi-skimmed milk, respectively using the fluidic sensor design.

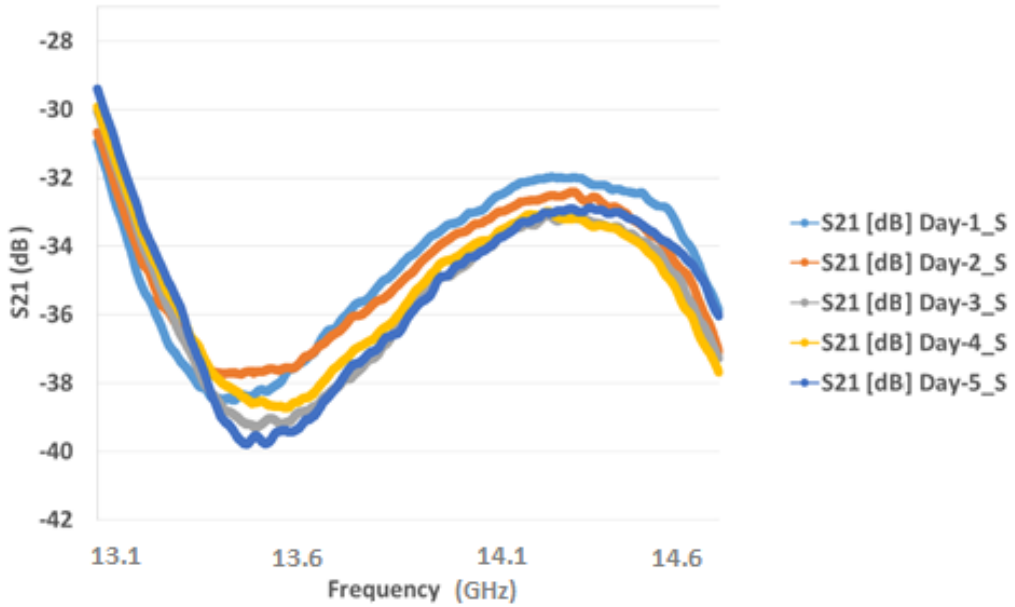


Figure 5.11 S_{21} vs. Frequency Day-1 to Day-5 for Skimmed Milk

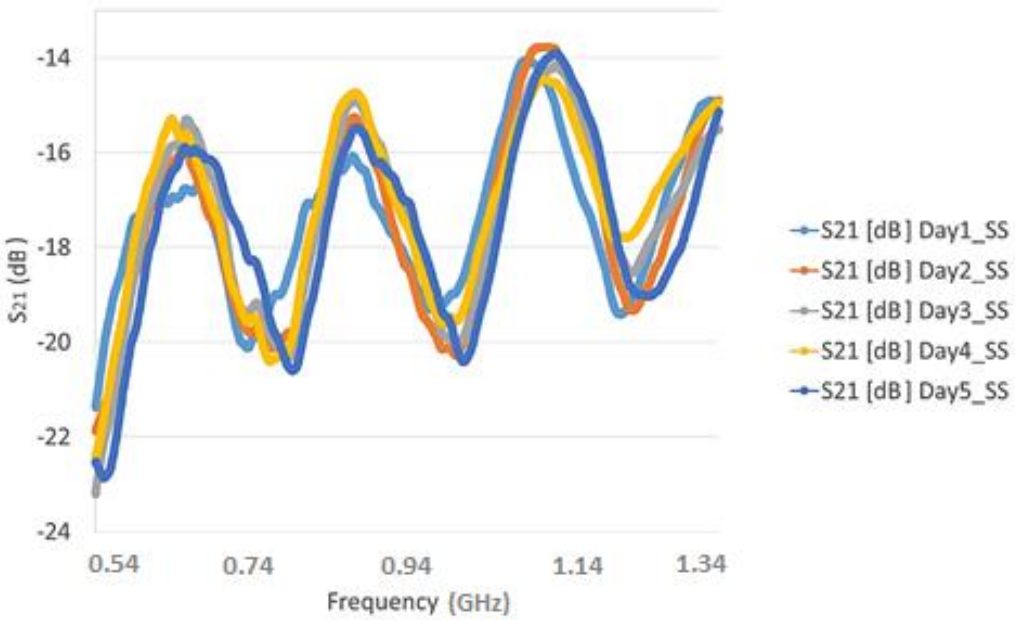


Figure 5.12 S_{21} vs. Frequency Day-1 to Day-5 for Semi-skimmed Milk

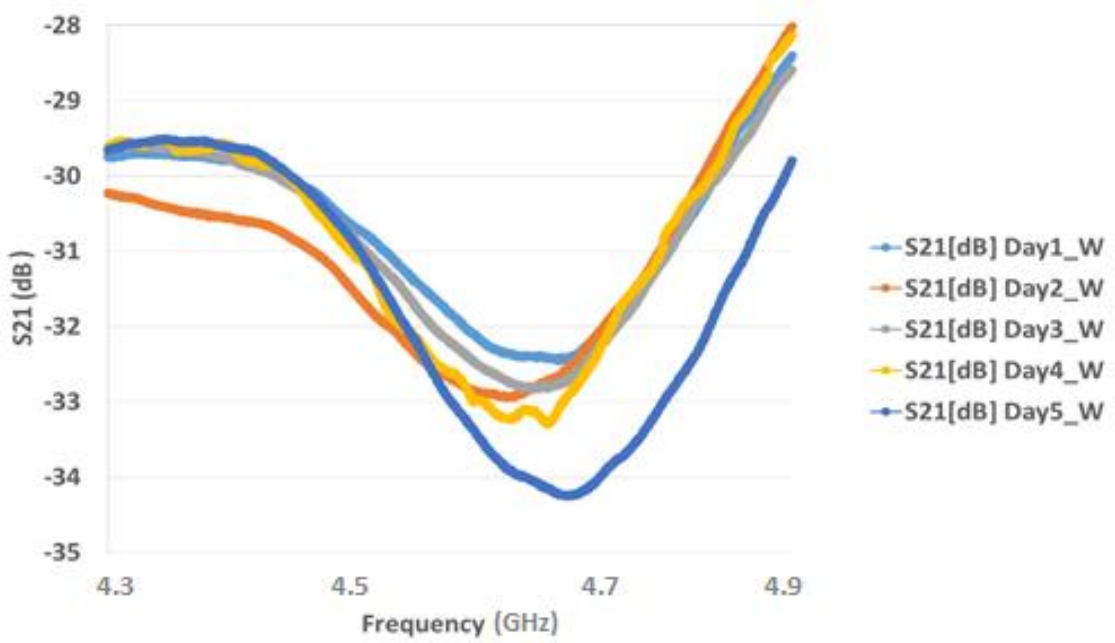


Figure 5.13 S_{21} vs. Frequency Day-1 to Day-5 for Whole Milk

It should be noted that for each case of milk type the expected and optimum results are seen at different frequency when we observe spoilage patterns through these graphs. Figures 5.13 shows Day-1 to Day-5 plots for S_{21} vs.

Frequency values for whole milk. The optimum results are seen at around 4.65 GHz were there is decrease in the transmission coefficient values, this confirms back to the fact that with spoilage there is water separation from milk solids and hence the scattering parameter values tend to decrease towards the value of deionised water scattering parameter values.

5.4 Summary

In this chapter experimental research work related to the spoilage of milk was covered using all the three different types of microwave sensors that are included in this project. Investigation was made for their individual responses and to check their feasibility in design and development of a milk testing system that can monitor and thereby help in maintenance of its quality in terms spoilage detection.

The biggest advantage of such microwave sensor system application is that the measurement are not only rapid but also give reliable results complimenting with

the expected trend and also confirming the fact that spoilage patterns in all milk types is predominantly similar, regardless of the compositional changes such as that in fat content.

Moreover, it should be noted that the IDE sensors are invasive in nature like most other existing techniques of practice involved in milk quality determination currently, whereas the hairpin resonator sensors do not require the sample content to be contaminated by directly exposing them to the physical contact of the sensor. Resonator cavities on the other hand takes a sample inside the test-tube for test measurements.

CHAPTER 6

CLASSIFYING MILK QUALITY BASED ON CONTENT

6.1 Introduction and Background

To design and develop a methodology with a system that can test the overall quality of pasteurised and homogenised milk types, in terms of their nutritional content values, it was important to understand the fundamental nature of the milk categories under consideration. Therefore, the three commercially predominant categories of milk, in the consumer market, namely — whole milk, skimmed milk and semi-skimmed milk were reviewed along with their spoilage patterns.

The Dairy Council, UK, categorises these three milk types based on the amount of fat content that they carry (The Dairy Council, 2016). Per 100 ml of given volume, skimmed milk should not have fat content exceeding the 0.3 g, whereas, whole milk should have a minimum of 3.5 g per 100 ml, and the semi-skimmed milk, fat content within the range between 1.5 g and 1.8 g per 100 ml of product.

6.2 Methodology and Measurements

Keeping the above definition in mind as well as the fact that the milk products are sold and labelled with reference to their fat content values, the primary goal of this portion of the test measurements was to be able to distinguish and classify each type of milk category on the basis of its fat component.

This section, explains the procedures adopted to carry out the milk quality analysis, made in terms of content values, again with the use of the same resonator cavity and Inter-digitated Electrode sensor designs, as in Chapter 5, to develop an idea of responses for each of them.

6.2.1 Sample Preparation

The sample preparations for the sensor B were made using the same milk as the previous measurements of spoilage (see Table 5.1); as there were no further data

points needed due to evidently visible distinct results for all three categories of milk samples based on their fat content.

However, in the case of the sensor A, the distinction was not quite evidently clear over a large span of frequencies, and hence to ensure more accuracy in results, it was necessary and equally important to have more data points for fat content values than just three. Hence, for a reliable plot of fat contents versus frequency value, another two categories were prepared by mixing two types of milk, respectively, in ascending order of fat content values as shown in Table 6.1. That is two more categories (total 5) of milk samples were derived by mixing 1:1 whole milk with semi-skimmed milk and similarly, 1:1 sample of skimmed milk mixed with the semi-skimmed milk.

Table 6.1 Two new milk categories derived by mixing milk types in ascending order of their fat contents for sensor A measurements

Typical Values (per 100 ml)	**Milk Categories [a]**				
	Skimmed	**Skimmed + Semi-skimmed**	**Semi-skimmed**	**Semi-skimmed + Whole**	**Whole**
Energy (kJ)	147 (35 kcal)	178 (42.5 kcal)	209 (50 kcal)	238.5 (57 kcal)	268 (64 kcal)
Fat (g)	0.1	0.95	1.8	2.7	3.6
Saturates (g)	<0.1	<0.6	1.1	1.7	2.3
Fibre (g)	0.0	0.0	0.0	0.0	0.0
Protein (g)	3.4	3.5	3.6	3.4	3.2
Carbohydrates	5.0	4.9	4.8	4.75	4.7
Sugars	5.0	4.9	4.8	4.75	4.7
Calcium (mg)	124.0	124.0	124.0	122.0	120.0

[a] Content values for each additional category are ideally assumed to be average of the corresponding values of both their constituents

Here the mixing of two categories of milk was done with volumetric sample preparation, using pipettes to ensure 50% volumes of each milk type being added together. The test-tubes were labelled as S1, S2,…, S5 for skimmed milk, SS1,

SS2,…, SS5 for semi-skimmed milk and W1, W2,…, W5 for whole milk, as abbreviated, respectively. Similarly the extra two categories with (semi-skimmed + skimmed milk) solution and (semi-skimmed + whole milk) solutions were labelled SSS1, SSS2, …, SSS5 and SSW1, SSW2, …, SSW5, respectively (see Figure 6.1) (Joshi *et al.*, 2017). Two more test tubes, one empty and one with deionised water, were added for comparative analysis.

Figure 6.1 Sample preparation for milk quality measurement based on its contents

6.2.2 Measurement conditions

Measurement conditions for these tests incorporating both the sensors were the same as cited in Table 5.2 and Table 5.3 for the sensor A and sensor B respectively, due to the same conditions and sensors being used for this set of test measurements. However, for the sensor A as explained in the previous section, two more categories of milk were introduced to get reliably accurate results as the spectral response in raw data was not showing clearly distinguished results.

Hence, from Table 5.2 the following new Table 6.2 could be derived, for the sensor A working to classify milk types based on their nutritional values, i.e. fat in this case. The following section discusses the results achieved with both of these sensors and gives gradation of milk samples based on their fat and protein contents.

Table 6.2 Measurement specifications for resonator cavity (sensor A): Based on contents

Specifications	Values
No. of Measurements	5 Types × 5 Samples × 2 Repetitions = 50 Iterations
Sample Size	15 ml (Polypropylene Test Tubes)
Supervision Period	5 days
Temperature Variations	22 °C ± 2 °C
Resonator cavity specifications	Diameter Φ = 130 mm (distance between ports) Height h = 20 mm (height of cavity)
Frequency Sweep	0.01 GHz – 15 GHz (10 KHz Measurement Bandwidth)
Channel Specifications	TOSM calibration / 0 dBm Base Power

6.3 Results and Discussion

6.3.1 Using Sensor A

Figure 6.2, classifies among the 5 various types of milk made from 3 categories of milk and labelled as S, SSS, SS, SSW and W, as explained earlier, based on their fat content. The figure illustrates a scattering parameter graph for S_{21} versus the fat content of the given milk sample. The linear regression with correlation value, R^2 = 98%, at the frequency value of 5.45986 GHz, was achieved. The selection of this particular frequency was made based on the data analysis done, and recorded spectral data looking for optimum results for correlation values as well as good separation between each two successive data point.

Similarly, Figure 6.3 shows the scattering parameter S_{21} values plotted against the protein content for the five milk categories. Here, unlike in the case of S_{21} vs. fat content graph, the linear regression gives correlation of, R^2 = 94.99% at 4.98968 GHz, which is due to relatively fewer variations in protein content as compared to that of the fat content (Joshi *et al.*, 2017). These results compliment the previous findings by others as discussed in the dielectric properties of milk in section 4.1, Chapter 4.

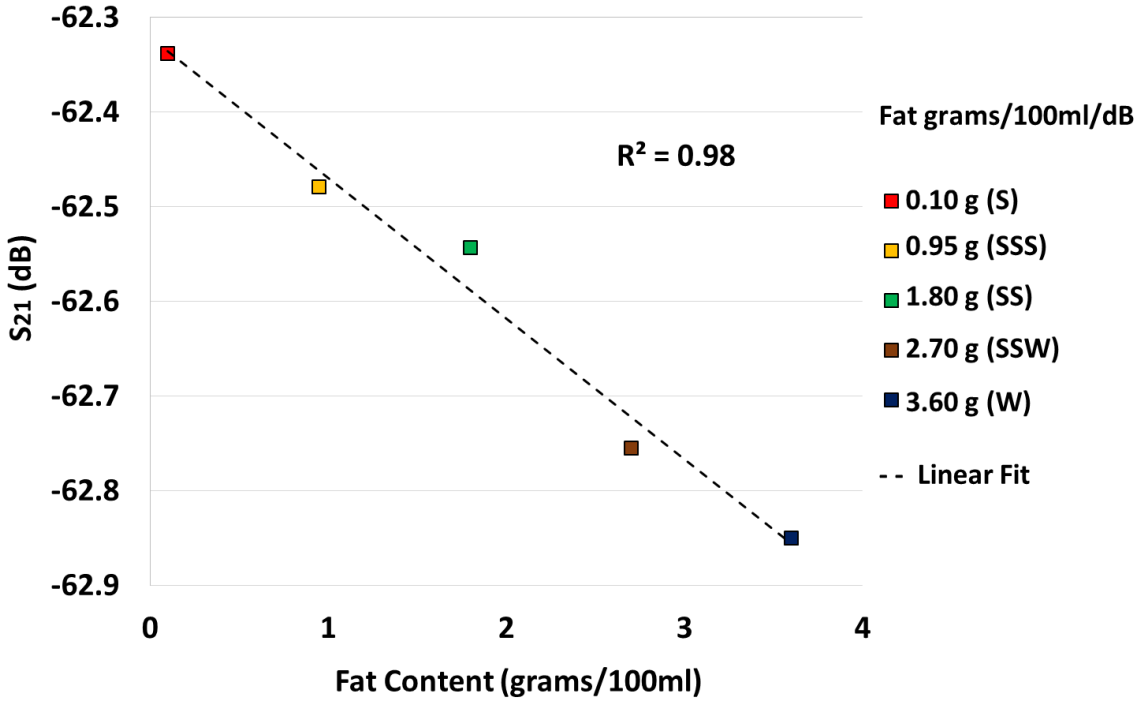


Figure 6.2 S_{21} vs. fat content graph using EM wave cavity sensor, at f = 5.45986 GHz

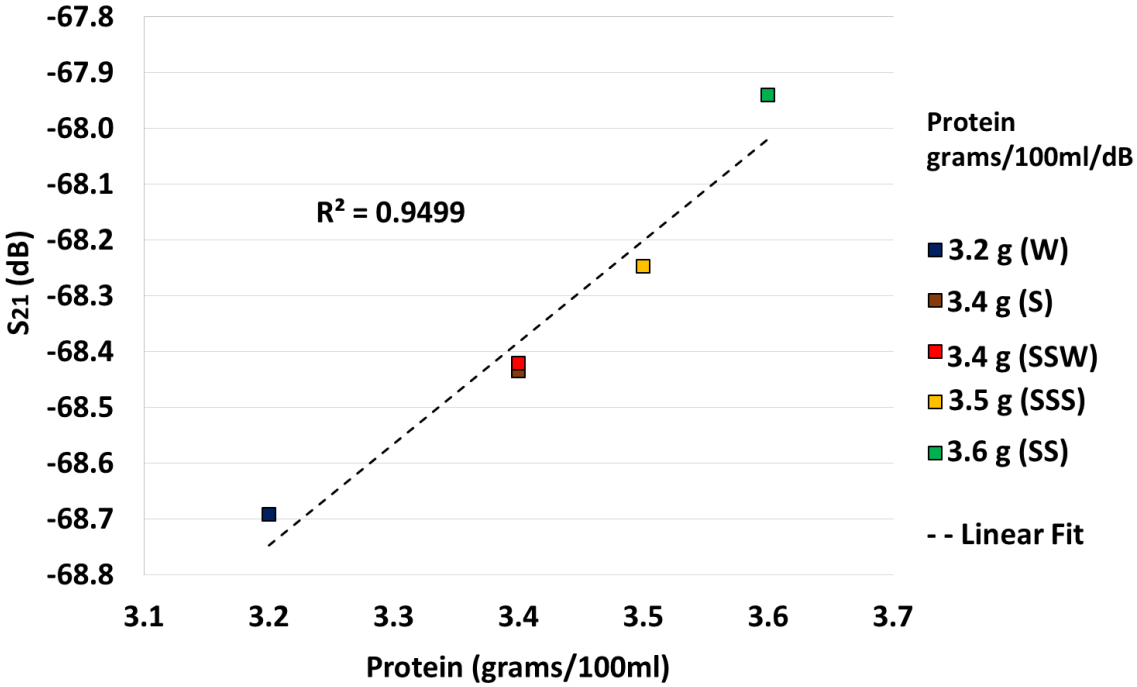
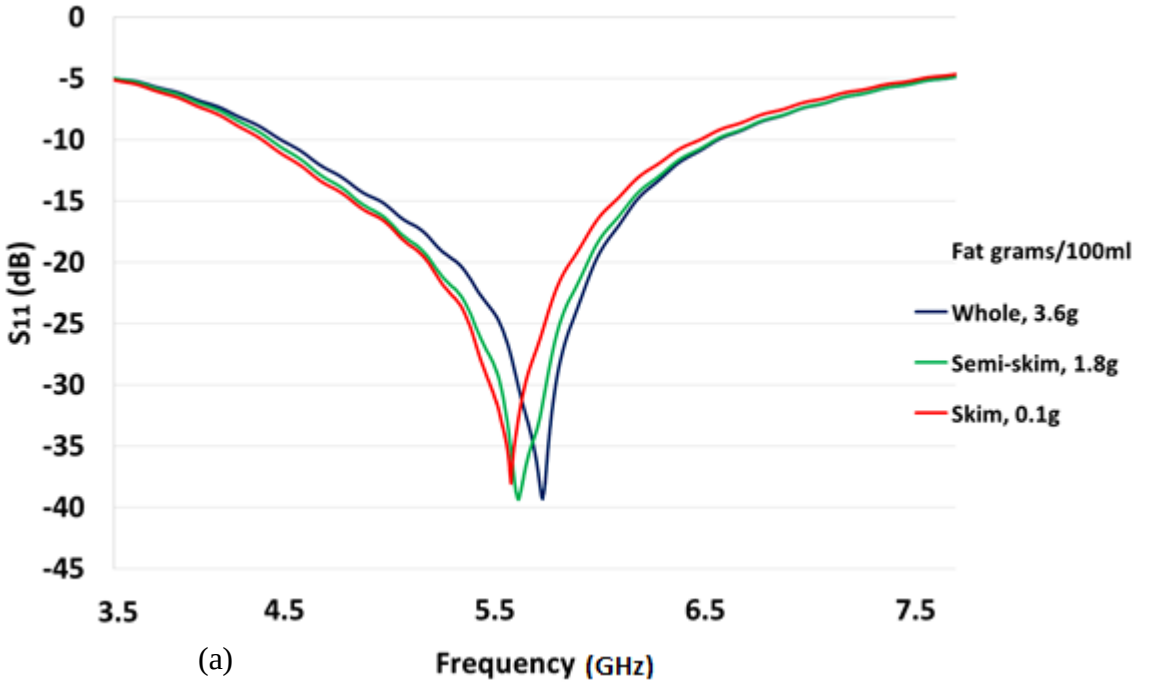


Figure 6.3 S_{21} vs. protein content graph using EM wave cavity sensor, at f = 4.98968 GHz

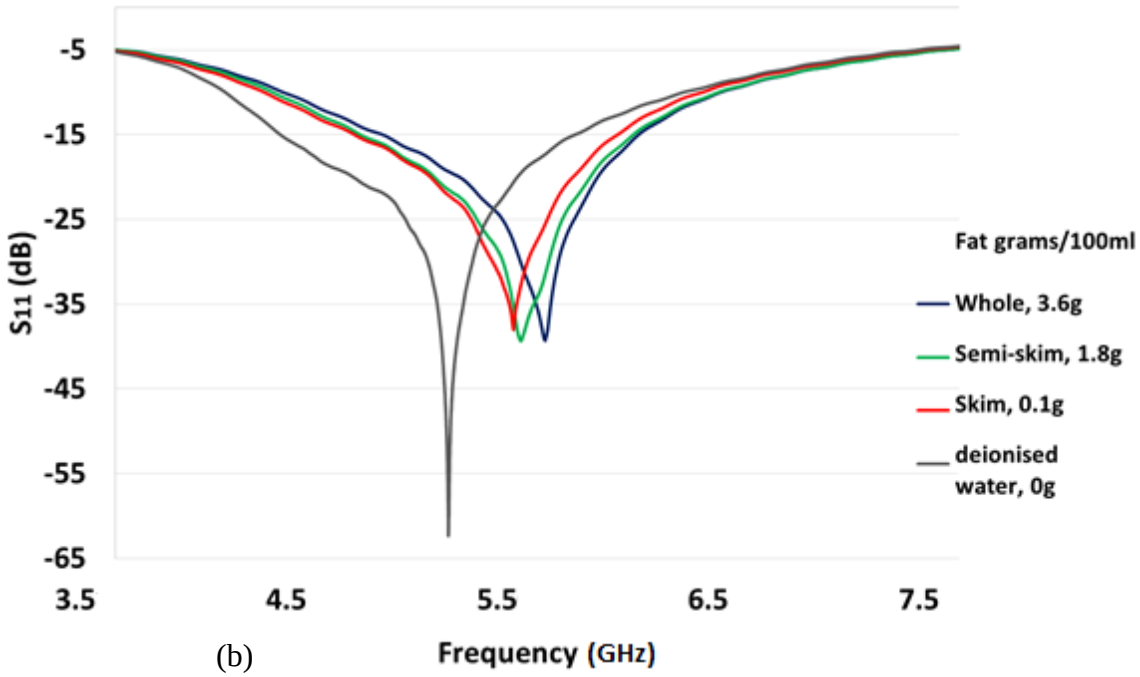
The numbers on the right, in Figure 6.2 and Figure 6.3 indicate the value of fat content and protein content respectively per 100 ml of milk sample, respectively.

6.3.2 Using sensor B

On the other hand, Figure 6.4(a) distinguishes among the three categories of milk using S_{11} spectral graph against the fat content for each type, using sensor B. We can see a clear frequency shift to the right as the fat amount increases. Whereas Figure 6.4(b) shows the same graphs with an additional plot of deionised water.



(a)



(b)

Figure 6.4 S_{11} vs. frequency graph using IDE sensor for: (a) three types of milk (b) three milk types and deionised water

When an MUT is placed close to an IDE, the sample proximity disturb the electromagnetic field, resulting in various shifts in resonance frequency.

6.3.3 Using Sensor C

The sensor C was able to distinguish among all three milk types as seen from Figure 6.5. Clear amplitude shifts and minor frequency shifts were seen in data analysis and plotted graph.

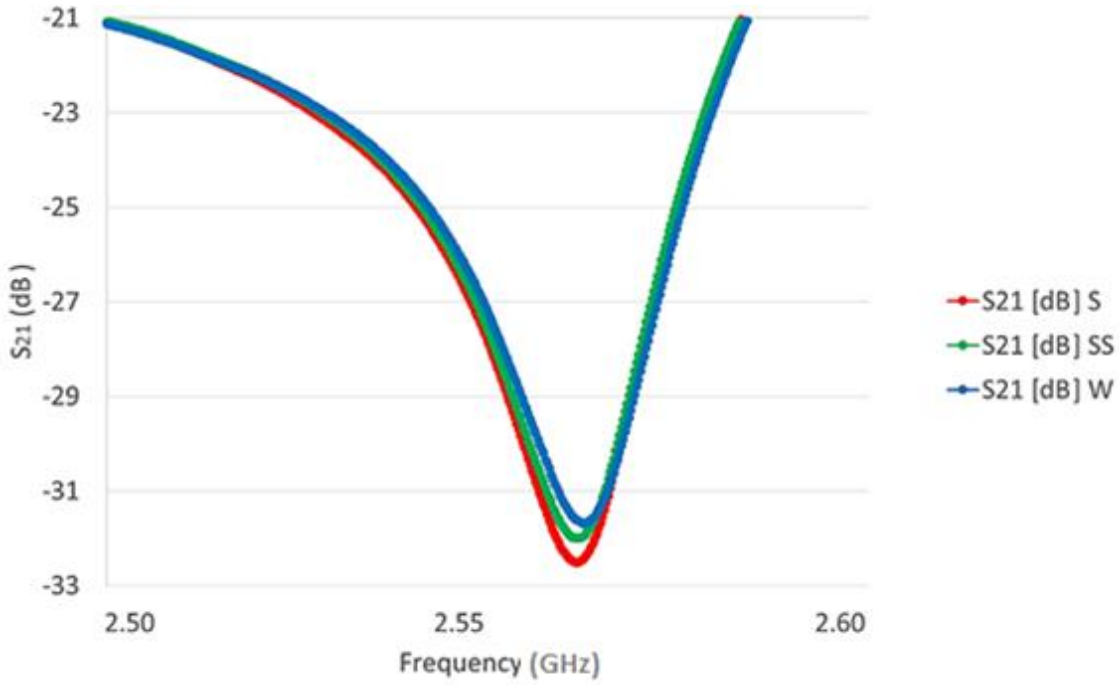


Figure 6.5 S_{21} vs. Frequency plot with hairpin resonator fluidic sensor Day-1

6.4 Summary

The biggest benefit of this novel method is, it does not only detect spoilage but also distinguishes well among the types of milk as can be seen from the results achieved. This technique is less complex, and a more real-time result oriented method than other methodologies previously explained in the literature review.

The primary characteristics of the 2-port sensor A was that it gave amplitude shifts at certain frequency for variations in milk types, whereas the IDE sensors primarily showed frequency shifts as the milk type under test changed from skimmed milk to semi-skimmed milk and from semi-skimmed milk to whole milk.

The sensor C gave minor frequency shift and amplitude shifts, both.

The following chapter discusses detection of adulteration in milk using the proposed fluidic sensor design as compared with the gold standard spectrophotometric methods in practice.

CHAPTER 7

DETECTION OF ADULTERATION IN MILK

7.1 Background

This chapter covers the third and final objective of the project aim, which is to determine the adulteration of milk, using EM wave spectroscopy. The adulterants can be any foreign particles that are not inherent components of milk, some of which can be harmless or ineffective when present under a certain threshold value. However, many adulterants can cause severe consequences if their presence is not identified within milk. As explained in section 1.4 of the thesis, milk adulteration can cause a wide range of food borne diseases - ranging from minor symptoms like abdominal cramps or fever to fatal diseases such as Haemolytic Uraemic Syndrome (HUS).

HUS results from E. coli infection, potentially leading to renal failure, specifically, in people with tempered immunity (Guntupalli *et al.*, 2007; News Desk, 2012). Hence, detecting such adulteration should be an integral part of the milk quality monitoring and control process. A study by the Food and Agriculture Organisation (FAO) of the United Nations shows that some contaminants can enter into the dairy chain even through the stage of milk processing and packaging or via deliberate means of adulteration, practised for the purpose of making commercial gains (Kenny, 2013).

The existing standard method uses spectrophotometry (Azad and Ahmed, 2016), as used primarily by many research labs worldwide including at the Department of Analytical Research and Quality of Food, in Ukraine and explained in the manual of methods of analysis of foods, Milk and Milk products, Food Safety and Standards Authority of India (FSSAI, 2012). The spectrophotometric method was used as a gold standard and replicated in the lab and the results achieved thereby were used to compare and validate the results obtained by the proposed novel methodology using microwave sensors in determination of Urea adulteration in milk, highlighting the effectiveness of using microwave spectroscopy. The results achieved here using a non-invasive, low profile and portable, electromagnetic

wave resonator sensor are promising and discussed at the end of this chapter. Here, in this chapter an application of a planar resonator, which acts as a fluidic sensor carrying the milk sample in a tube for online determination the urea content, is very successfully demonstrated, followed by detection of detergent presence in water used as a cleaning agent for milk tankers have been tested, determined and results discussed at the end.

7.2 Methodology and Measurements

In the first part, spectrophotometry with para-dimethyl amino benzaldehyde (DMAB) was used for estimation of urea in milk after precipitation of milk proteins with the help of trichloroacetic acid (TCA), was used as a gold standard. In the second part of the experiments, the proposed novel method was used to determine the presence of urea in milk samples using microwave spectroscopy using a planar resonator sensor.

Eight milk samples were prepared, for both parts of the tests, with varying urea concentrations. The manual spiking was done to enable testing of the sensor's ability to detect urea in the milk samples. Volumetric sample making, was achieved using the standard and following equation (6.1):

$$C_1 V_1 = C_2 V_2 \tag{7.1}$$

where, C_1 = 14 mg/10 ml, the concentration of urea in milk stock solution starting from the highest concentration of 1400 ppm of urea in skim milk. All the other varying concentrations (C_2) of spiked urea were then achieved as shown in Table 7.1. For all sample preparations precision balance KERN® PCB100-3 was used.

Table 7.1 Milk samples spiked with 8-fold urea concentration

Test-Tube Sample Number	Spiked Urea C_2 (mg/10ml)	Skim milk solution, V_2 ml	Urea in skim milk stock solution[1], V_1 ml
1	0	10	0
2	2	8.5715	1.4285
3	4	7.1430	2.8570
4	6	5.7145	4.2855
5	8	4.2855	5.7145
6	10	2.8570	7.1430
7	12	1.4285	8.5715
8	14	0	10

[1] C_1 = 1400 ppm (14 mg/10 ml)

7.2.1 Spectrophotometry (Gold-standard)

7.2.1.1 Sample Preparation

To prepare p-Dimethyl Amino Benzaldehyde (DMAB) solution, 1.6 g DMAB (Sigma Aldrich, 109762) was dissolved in 100 ml ethyl alcohol (Ethanol – Sigma Aldrich, 02860) and then 10 ml concentrate Hydrochloric (HCl) acid was added. This reagent is stable for one month. A new standard curve was prepared with each new batch of reagent as required.

A Phosphate buffer with *pH 7.0* was prepared by dissolving 3.403 g anhydrous potassium dihydrogen orthophosphate - KH_2PO_4 (BDH AnalR, 10203) and 4.355 g anhydrous dipotassium monohydrogen orthophosphate - K_2HPO_4 (Sigma Aldrich, 795496) separately in 100 ml of deionised water. These solutions were, then combined and, diluted to 1 litre with deionised water. The pH of the solution can be adjusted to 7.0 by adding more K_2HPO_4 (if the pH were lower than 7) or by adding KH_2PO_4 (if the pH were higher than 7) - adding either of the two by small parts and mixing the entire volume before testing pH, as required.

Trichloroacetic Acid (TCA), 24% w/v solution was freshly prepared by dissolving 24.0 g TCA in deionised water and total volume made up to 100 ml.

Urea standard stock solution (5 mg/ml) was prepared with 5±0.001 g urea (Sigma Aldrich, U5378) was dissolved and diluted with 1 litre of deionised water. This can be adjusted as per required volume.

7.2.1.2 Instrumentation

In the first part of measurements, spectrophotometry was implemented using a Jenway Spectrophotometer Model 7315. The pH values were determined using a Hanna pH 213 Microprocessor pH Meter. Centrifugation, for the precipitation of proteins, was achieved using a Sigma 3-16PK Laboratory Centrifuge. Filtration was carried out using a Welch® 2511 Dry Vacuum Pump/Compressor.

7.2.1.3 Procedure

Standard conventional procedure, was replicated in the first part of the experiments, which uses spectrophotometry, as used at the Department of Analytical Research and Quality of Foods, in Ukraine, and many other research

organisations as presented in FSSAI (2012). To prepare standard curve calibration, solutions were made by adding necessary volumes of phosphate buffer and of urea solution. 10% w/v skimmed milk standard solution (Sigma Aldrich, 70166) was prepared, as per the product data sheet. Hence, 10g of skimmed milk powder was dissolved in deionised water (solvent), making the final volume of solution up to 100ml.

Each of the eight milk samples of 10 ml in total was weighed (in grams), and added with 10 ml of 24% TCA solution, to precipitate the proteins, and weighed again (in grams). These samples then were subjected to centrifugation at 4,500 rpm for 40 minutes. Each obtained sample was then filtered out using Whatman™ grade 42 filter papers (Ashless - without nitrogen) and funnels. 5 ml of filtrate of each sample was then added in eight separate test tubes**.**

When tubes with filtrate of spiked urea milk samples were ready, 5 ml of DMAB solution was added in each of the eight test tubes. Test tubes were thoroughly shaken and left to stand for 10 minutes at room temperature. First, the cuvette (cell) for the blank sample, made of 5 ml buffer and 5 ml DMAB, was measured as a reference (zero) point in the spectrophotometer, at 420 nm wavelength, and then one by one, the cuvettes for each calibration solution and then for each milk filtrate sample were measured. For minimising errors resulting from reuse of cuvettes, each measurement in the spectrophotometer was carried out using one-time use disposable cuvettes.

Table 7.2 Calculation of masses to determine dilution factors in spiked milk

Sample No.	M1 (g)	M2 (g)	M3 (g)	[M2-M1] M4 (g)	[M3-M1] M5 (g)	M6 (g)	[M6-M1] M7 (g)	[M5-M7] M8 (g)	Dilution Factor (M5/M8)
1	13.813	23.726	34.912	9.913	21.099	14.836	1.023	20.076	1.051
2	13.889	23.773	34.852	9.884	20.963	14.885	0.996	19.967	1.050
3	13.709	23.475	34.560	9.766	20.851	14.701	0.992	19.859	1.050
4	13.163	22.624	34.717	9.461	21.554	14.050	0.887	20.667	1.043
5	13.784	23.491	34.651	9.707	20.867	14.712	0.928	19.939	1.047
6	13.828	23.597	34.496	9.769	20.668	14.667	0.839	19.829	1.042
7	13.721	23.556	34.670	9.835	20.949	14.681	0.96	19.989	1.048
8	13.586	23.149	34.270	9.563	20.684	14.648	1.062	19.622	1.054

M1 = mass of empty centrifugation tube;
M2 = mass of centrifugation tube with 10 ml of the milk sample;
M3 = mass of centrifugation tube with 10 ml of the milk sample and with 10 ml of 24% TCA;
M4 = mass of 10 ml of the milk sample;
M5 = mass of 10 ml of the milk sample with 10 ml of 24% TCA;
M6 = mass of centrifugation tube with precipitated protein (after centrifugation and filtering);
M7 = mass of precipitated protein; and M8 = mass of supernatant;

Figure 7.1 8-milk samples in the centrifuge grouped in two sets of equal masses

Table 7.2 gives calculation of masses to determine the dilution factor. Based on these values, the milk samples were grouped into two sets of equal masses for centrifugation as shown in Figure 7.1.

The milk samples after centrifugation stage with precipitated proteins are shown in Figure 7.2.

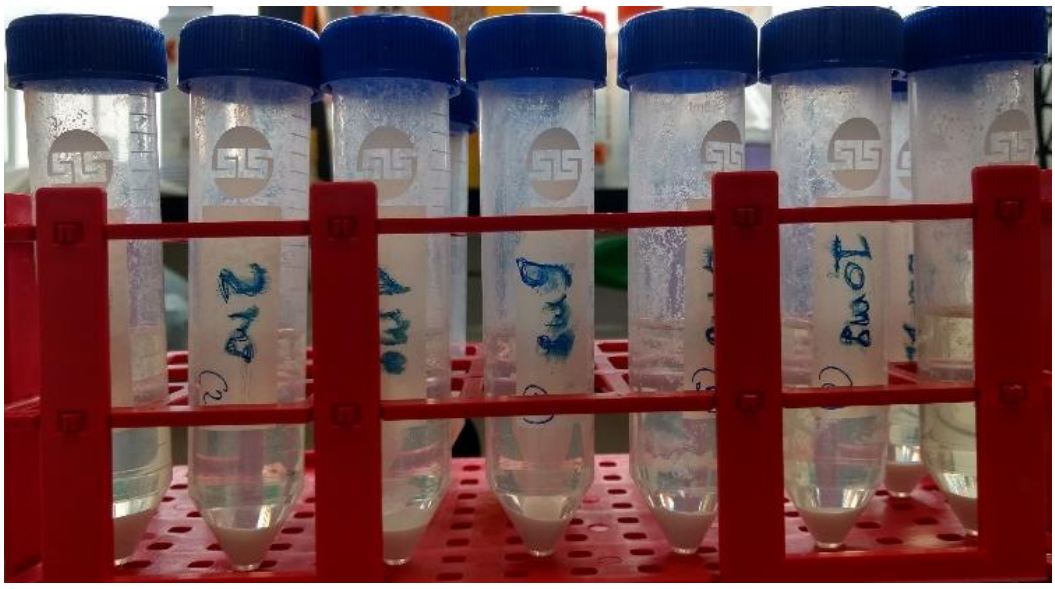


Figure 7.2 Milk samples with precipitated protein after centrifugation

The filtration setup, to separate milk proteins from remaining milk sample is demonstrated in Figure 7.3.

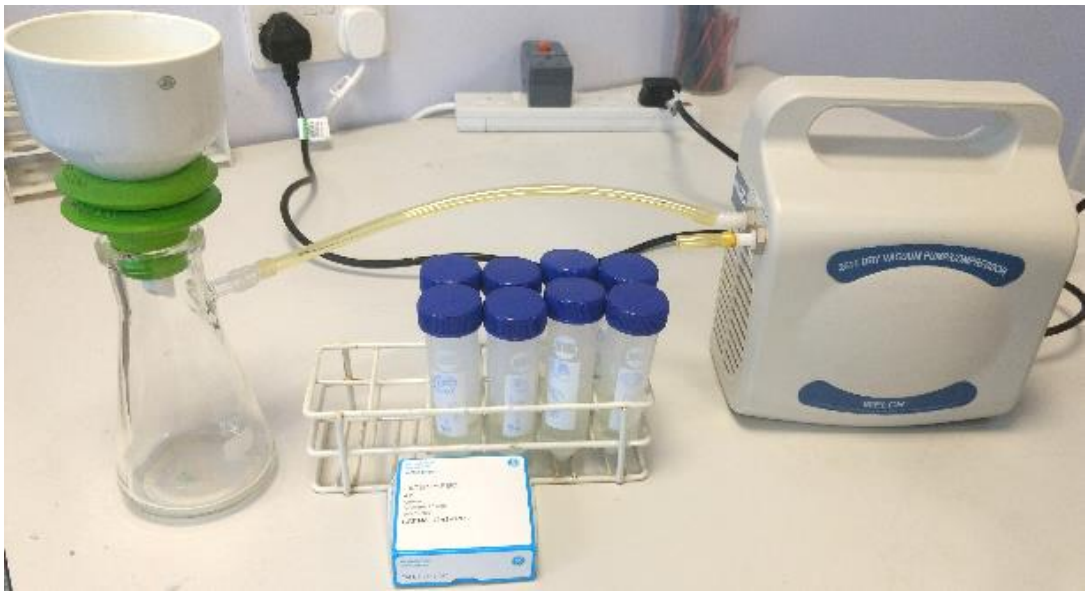


Figure 7.3 Filtration of precipitated protein from milk samples

The samples added with colour reagent (DMAB) ready for spectrophotometry are illustrated in Figure 7.4.

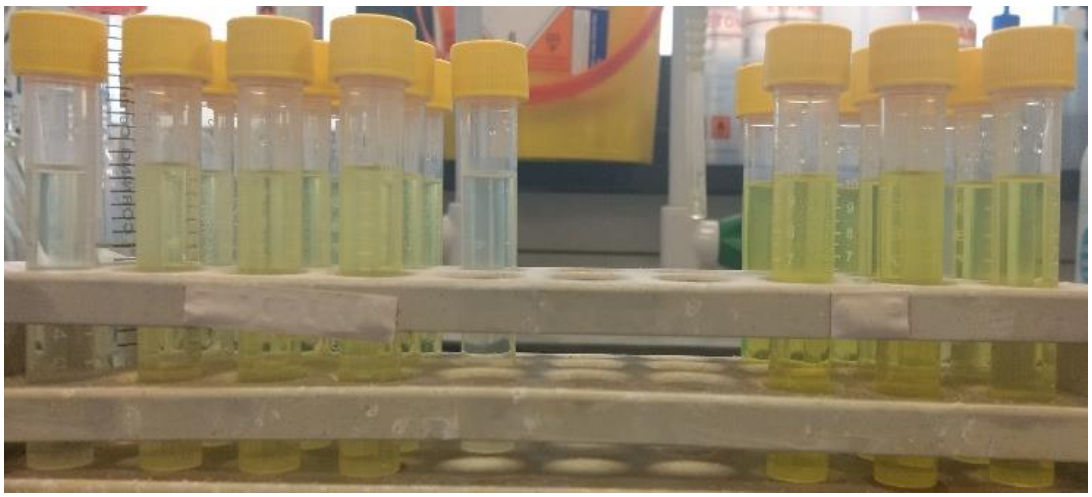


Figure 7.4 Samples added with colour reagent for spectrophotometry

7.2.2 Microwave Spectroscopy

In this proposed method, use of a planar resonator, acting as a fluidic sensor, was made to determine the urea adulteration in milk. The experimental set-up is illustrated in Figure 7.6.

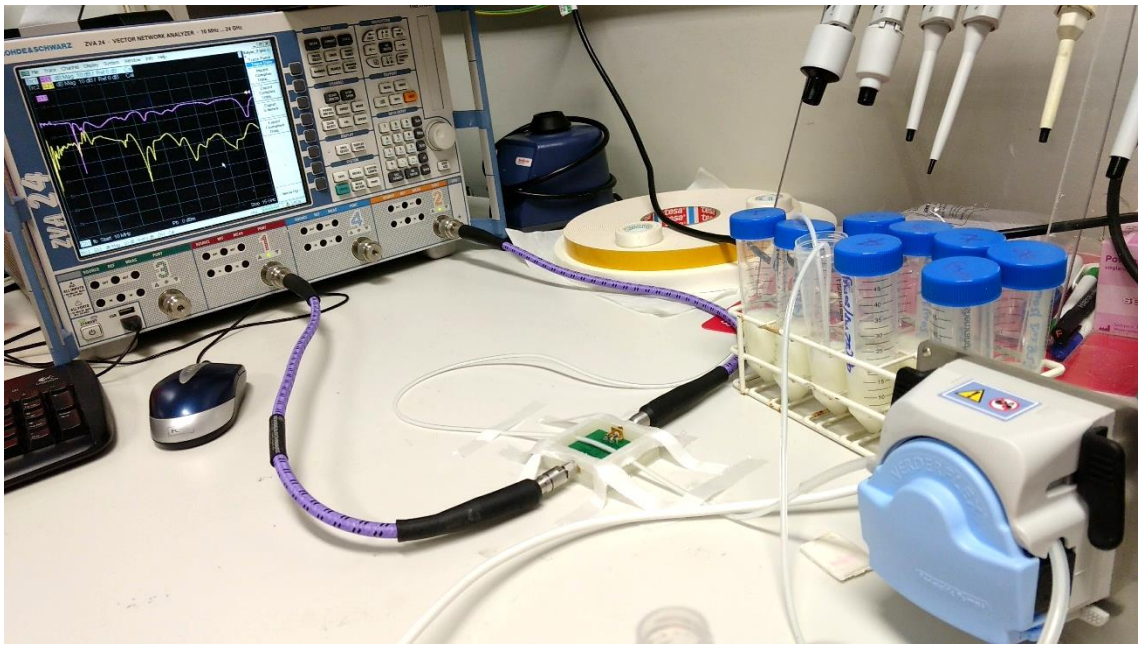


Figure 7.5 Experimental setup of determining urea detection in milk using Microwave sensor

Whereas, the EM wave sensor carrying the fluid sample under test is shown in Figure 7.6.

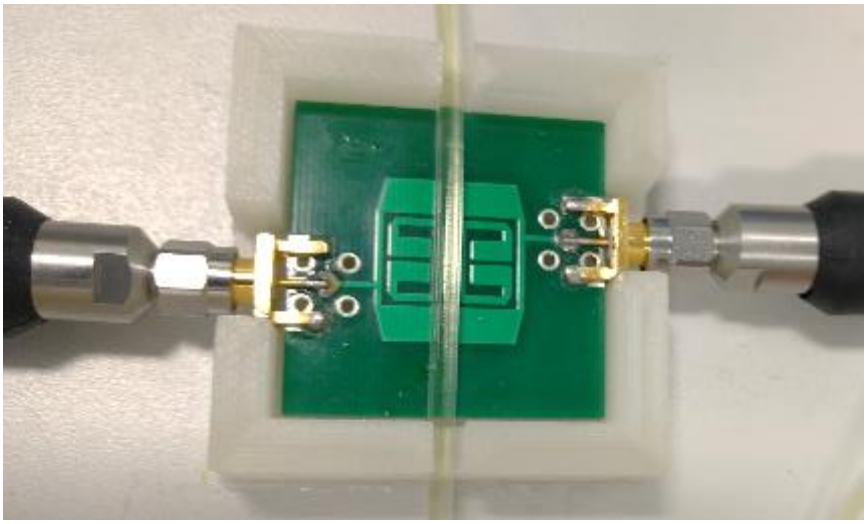


Figure 7.6 The coated planar resonator sensor with tube carrying fluid sample

7.2.2.1 Instrumentation

For the second part where the proposed microwave spectroscopy technique was implemented, a fluidic sensor design was optimised using ANSYS High Frequency Structure Simulator (HFSS) software followed by its fabrication.

The spectral signatures, in terms of scattering parameters were recorded by R&S® ZVA vector network analyser at 10 MHz to 15 GHz frequency range, with 0 dBm base channel power and 10 KHz bandwidth. To carry the milk sample through the fluidic sensor, a peristaltic pump Verderflex® operating on 12 V DC supply was used.

7.2.2.2 Procedure

The sensor design was optimised so that a tube carrying the sample under test can be used for non-invasive application of microwave spectroscopy. Five repetitions for each of the 8-points of urea spiked milk samples were made to achieve good robustness of obtained measurement data. All data were then aggregated and data analysed to identify the optimum frequency out of the full sweep of the spectrum. Room temperature was maintained during the time measurements were carried out.

7.3 Results and Discussion

7.3.1 Using Spectrophotometry (Gold-standard)

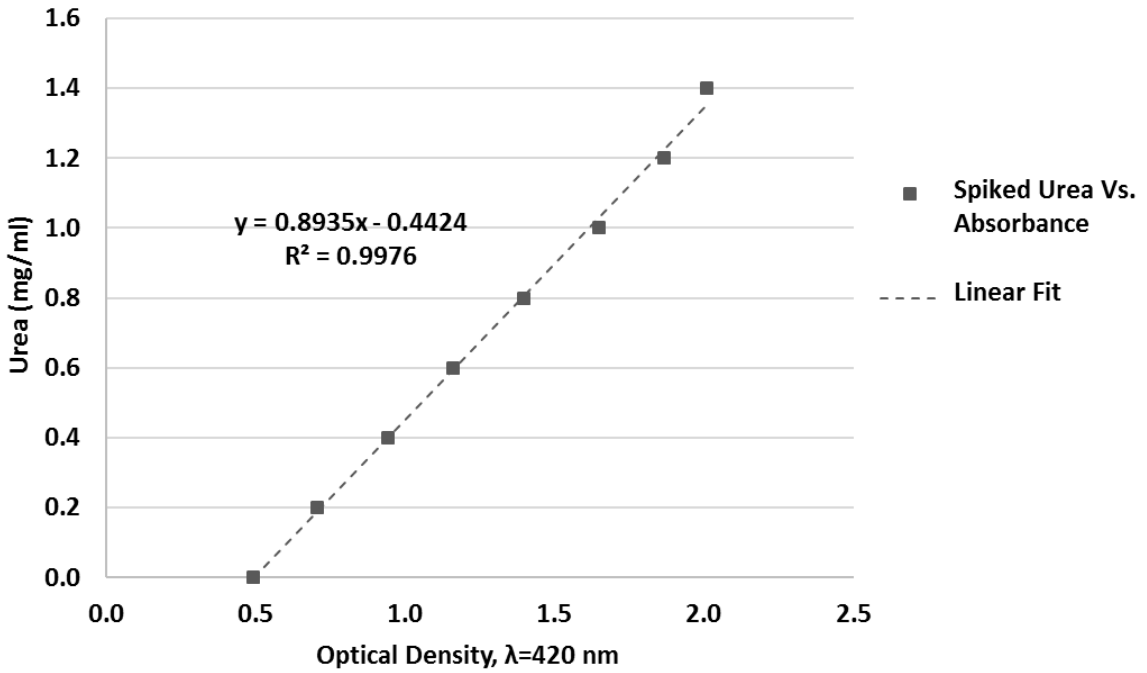


Figure 7.7 Skimmed milk samples spiked with urea as detected in spectrophotometer

Figure 7.7 identifies 8-point milk samples with varying concentrations of spiked urea within skimmed milk samples, with spectrophotometry. The gold standard as can be seen from the graph shows a very good linear regression with 99.76% of correlation among the 8-milk samples spiked with urea content in ascending order of concentrations.

7.3.2 Using Proposed Methodology (EM Wave Spectroscopy)

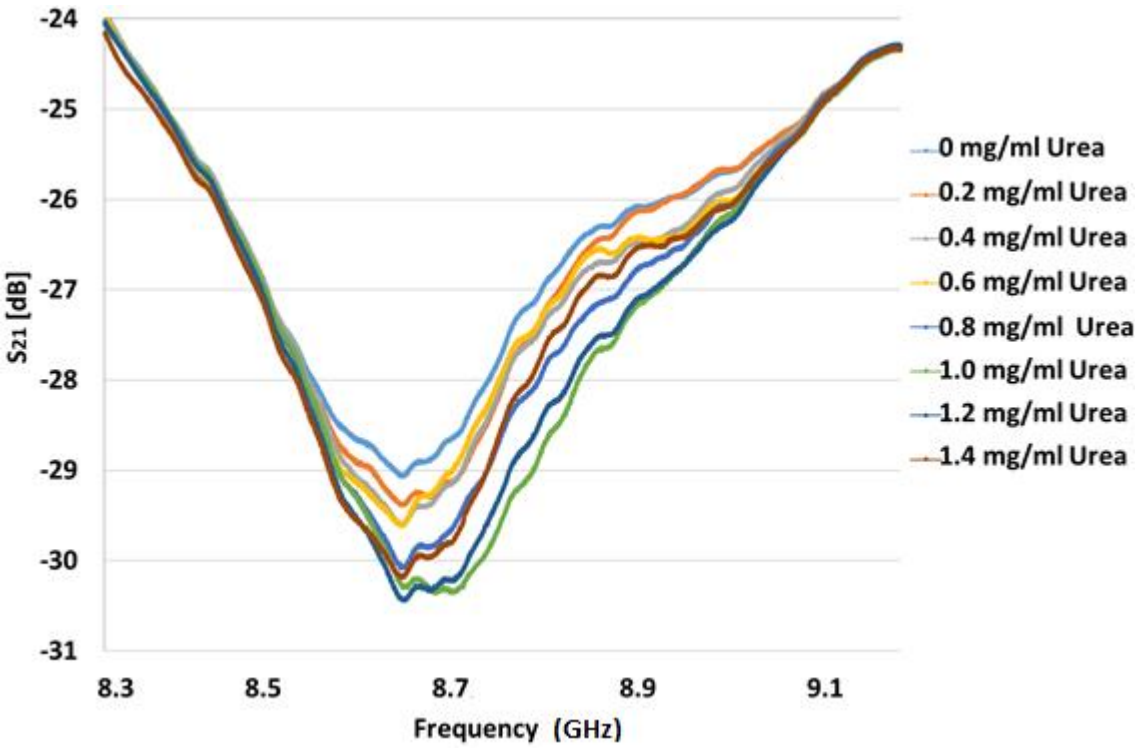


Figure 7.8 S_{21} vs. frequency plot for all spiked milk samples with urea between 8.3 GHz to 9.1 GHz

Figure 7.8 demonstrates the scattering parameter S_{21} (dB) vs. urea content (mg/ml) graph, over the span of 8.3 GHz to 9.1 GHz. From further data analysis the best response, using EM wave spectroscopy, giving distinctive results for varying urea concentrations was achieved at 8.5678 GHz frequency with a linear correlation of up to 96.18%, as further illustrated in Figure 7.9.

It should be noted that the results shown here are exclusive to spiked urea added manually to check the feasibility of the proposed model and therefore do not reflect any endogenous urea inherent to the prepared milk samples using skimmed milk powder. Although this method and all tests were done using

skimmed milk samples prepared from a standard batch of milk powder (Sigma Aldrich, 70166), it is evident that the same protocols are applicable to any milk type provided a standard protocol is followed.

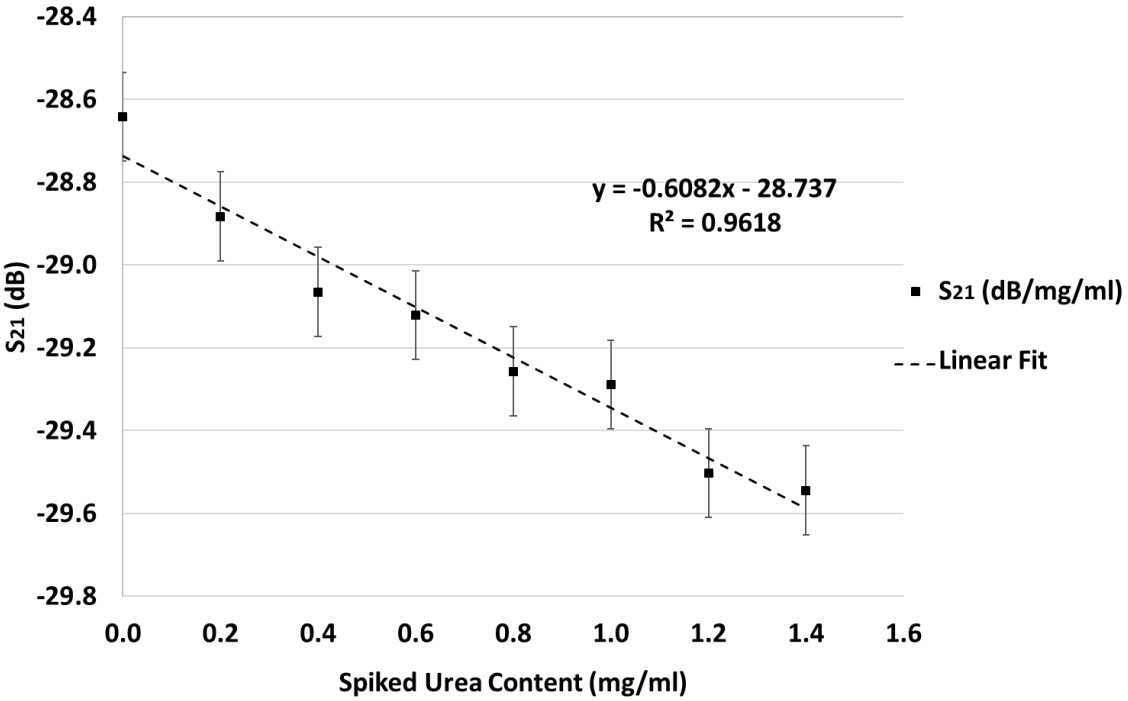


Figure 7.9 Precipitated milk samples spiked with urea adulteration, graded at 8.5678 GHz frequency using EM wave spectroscopy

Figure 7.10 shows the results achieved with direct urea spiked milk samples. Here, it is important to note that the data points are reduced to 7-points as the sensor undergoes limit of detection (LOD) and shows saturation beyond 1200 ppm.

However, this proposed technique eliminates tedious and cumbersome procedural steps and reagent preparation and time-restricted methodology as achieved in the gold standard with 99.22% linear correlation offering a real-time solution to the urea adulteration problem as faced by the dairy industry. The following section explains methodology involving detergents detection as cleaning agents.

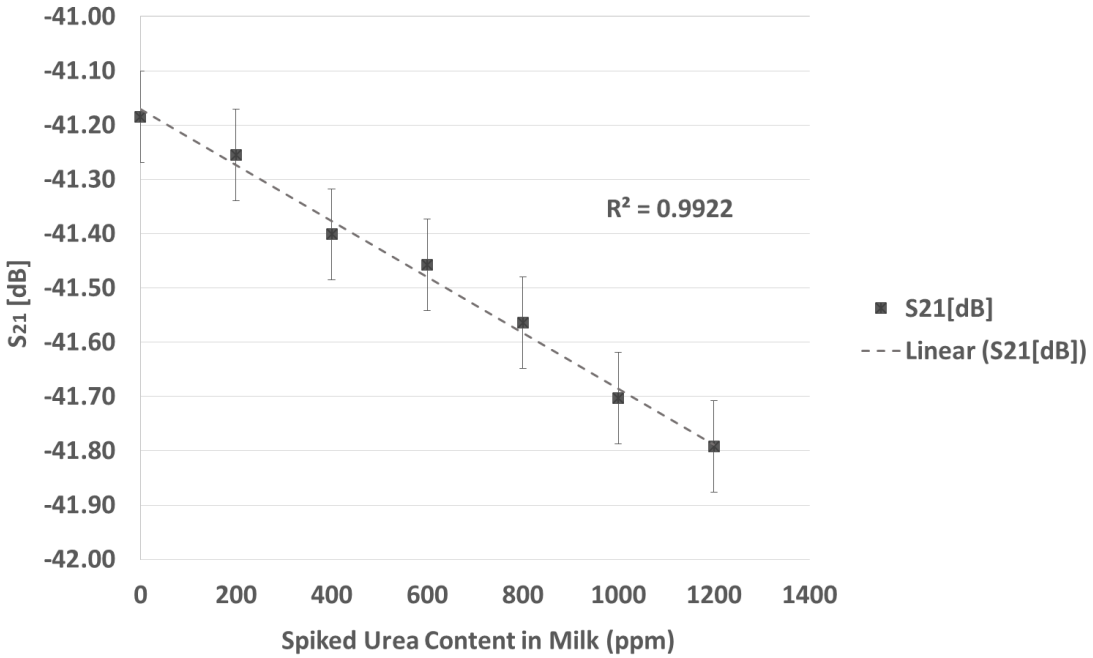


Figure 7.10 Urea spiked milk samples directly measured with hairpin resonator sensor

7.4 Detection of Detergents Present as Cleaning Agents

To check and distinguish among the residual concentrations of various Alkali (Liquid Gold) and Acidic (Acid Descaler) Solutions used as detergents in the dairy industry to clean up the milk storage vessels such as tankers, the following test conditions were observed for measurements as shown in Table 7.3. Table 7.4 shows 8-fold sample preparation values for both the detergents, acidic and alkali.

Table 7.3 Measurement Conditions for Detection of Detergent Presence in Milk

Sample Making	8-point Calibration
Methodology	Electromagnetic Wave Sensing
Liquid Detergents (8-samples each)	**A.)** Acid Descaler (acidic) **B.)** Liquid Gold (alkali)
Sensors	**i.)** Sensor A (2-ports) **ii.)** Sensor B (1-port) **iii.)** Sensor C (2-ports)
Frequency Sweep	10MHz to 15GHz
Base Power	10 dBm
Temperature	20º C
Repetitions	3 times
Control Medium	Deionized Water (DW)

Table 7.4 8-fold samples of Acid Descaler and Liquid Gold

Sample Label		
Acid Descaler	**Concentration (%)**	**Liquid Gold**
AD1	10	LG1
AD2	8	LG2
AD3	6	LG3
AD4	4	LG4
AD5	2	LG5
AD6	1	LG6
AD7	0.5	LG7
AD8	0.1	LG8
DW	0	D

7.4.1 Using Sensor A

In this case, 15ml test tubes were used as the sample size for measurements. Figure 7.11 and Figure 7.12 show two major distinctive curves within the S_{21} Vs. frequency plot of 8-point calibrated samples for Acid Descaler liquid solution separately magnified for the further detailing, respectively. Notice that the characteristics is reversed from one to the other.

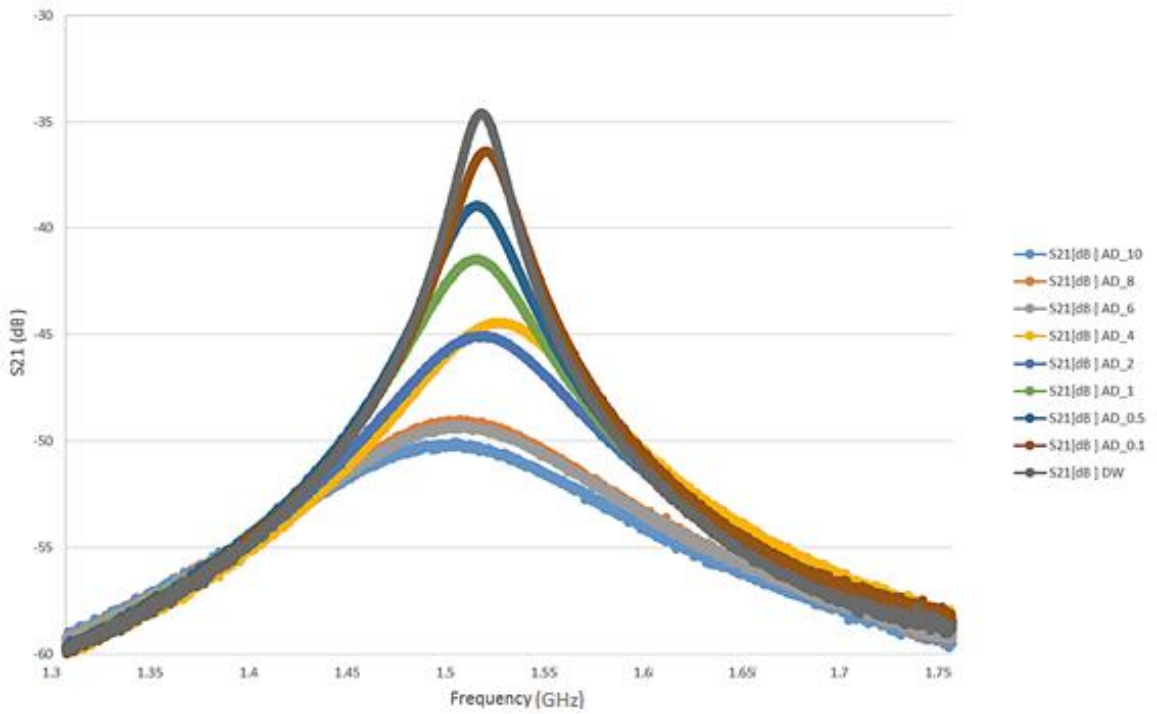


Figure 7.11 S_{21} Plot centred at 1.5 GHz for Acid Descaler 8-fold samples.

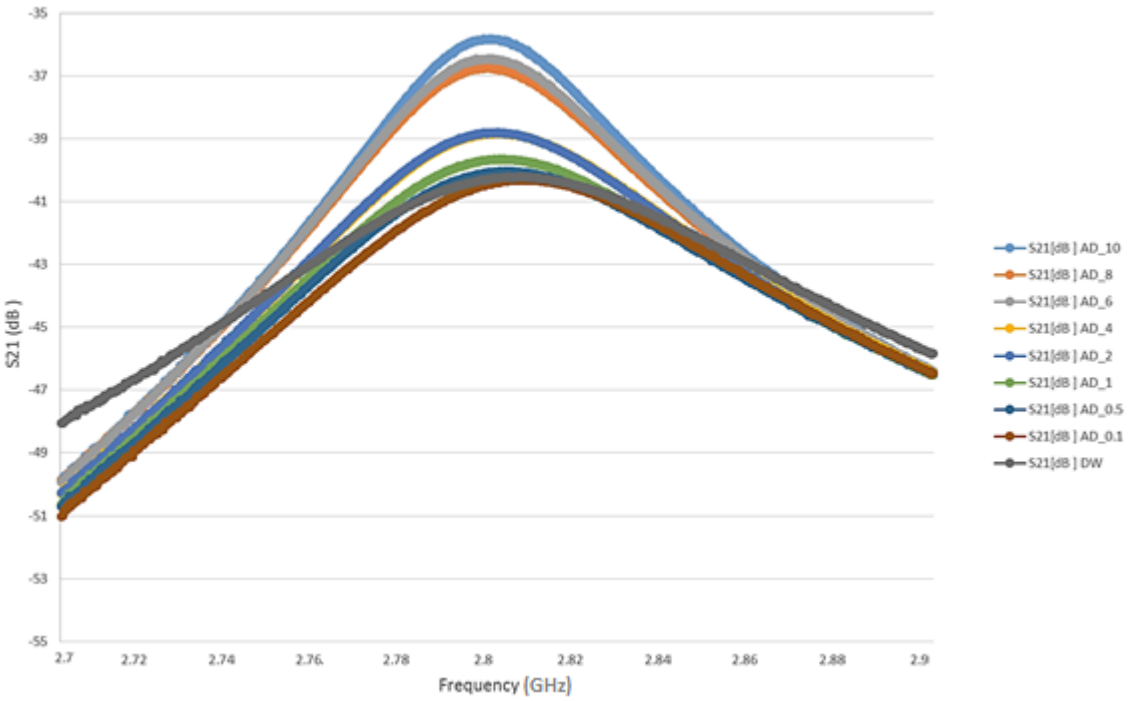


Figure 7.12 S_{21} Plot centred at 2.8 GHz for Acid Descaler 8-fold samples.

Similarly, Figure 7.13 and Figure 7.14 show two major distinctive curves for 8-point calibrated samples of Liquid Gold solution, located at around 1.5 GHz and 2.8 GHz, respectively.

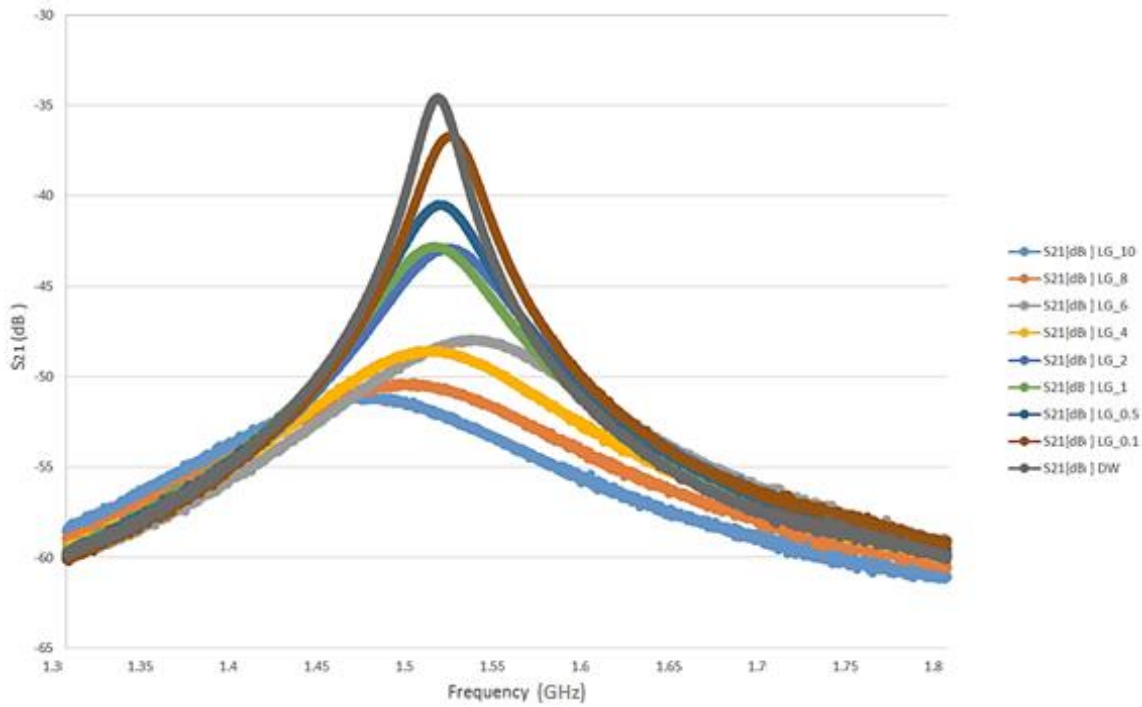


Figure 7.13 S_{21} Plot centred at 1.5 GHz for Liquid Gold 8-fold samples.

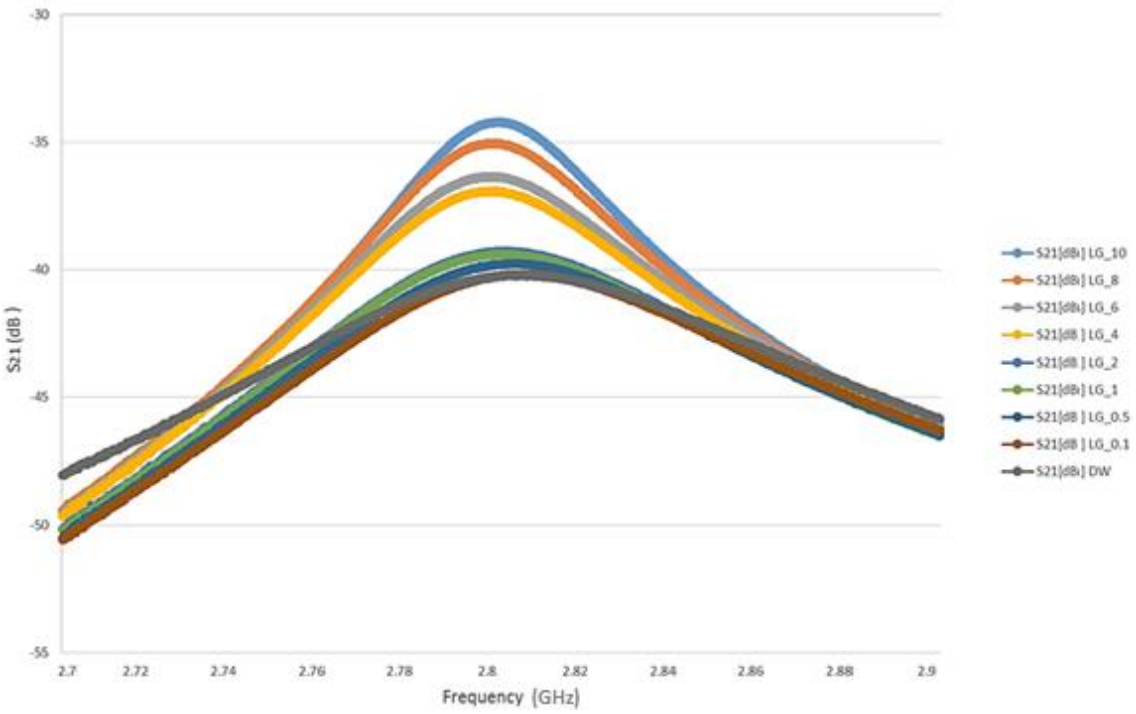


Figure 7.14 S_{21} Plot centred at 2.8 GHz for Liquid Gold 8-fold

Note that the same inverting of characteristics is seen in the Liquid Gold as it was seen in Acid Descaler, with the only difference being the centre radiation frequencies, the reason being the former is Acidic in nature and the latter is an Alkali.

Both the plots for S_{21} of Acid Descaler and Liquid Gold, if compared together, are quite similar in terms of characteristics with each other. In both cases, frequencies of interest remain 1.5GHz and 2.8GHz.

7.4.2 Using Sensor B

In the case of the sensor B, the sample size used was 500µl held within the well boundary around the sensor electrodes. Figure 7.15 and Figure 7.16 show clear classification of varying concentrations of each detergent active ingredient effectively in S_{11} Vs. frequency graphs, respectively, for Acid Descaler (AD) and Liquid Gold (LG), within the given range of frequency 0 to 1.5 GHz.

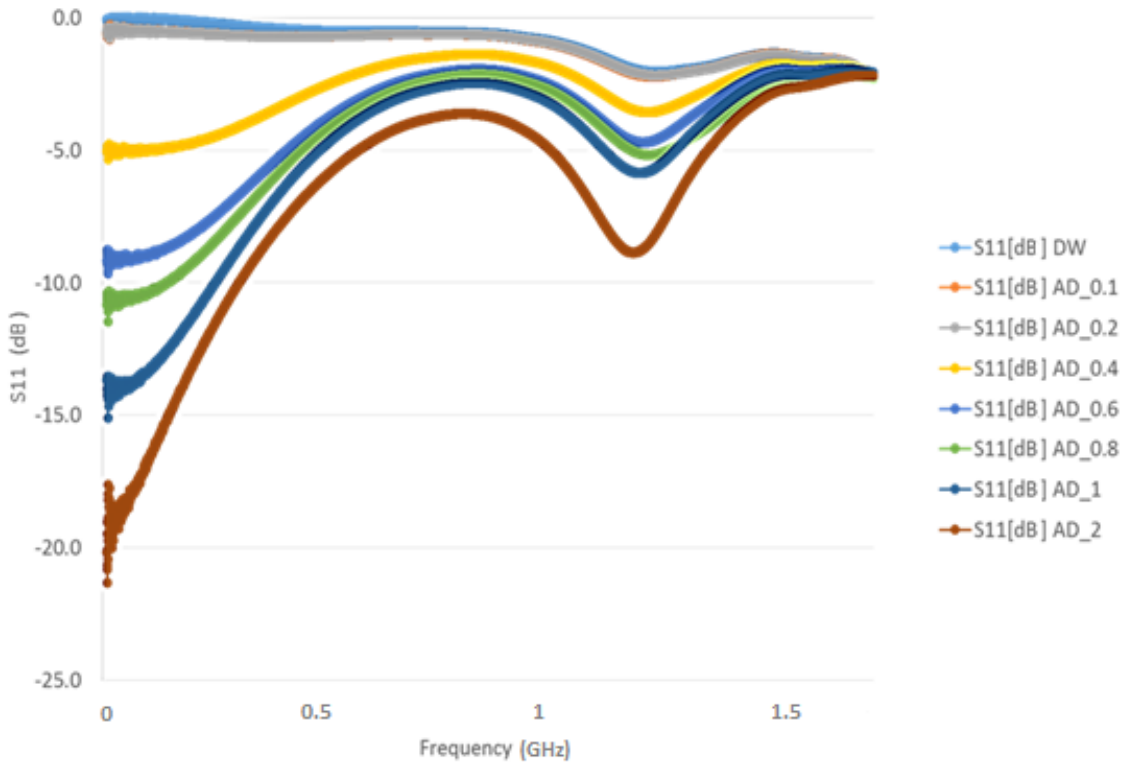


Figure 7.15 S_{11} vs. Frequency Acid Descaler 8-fold samples

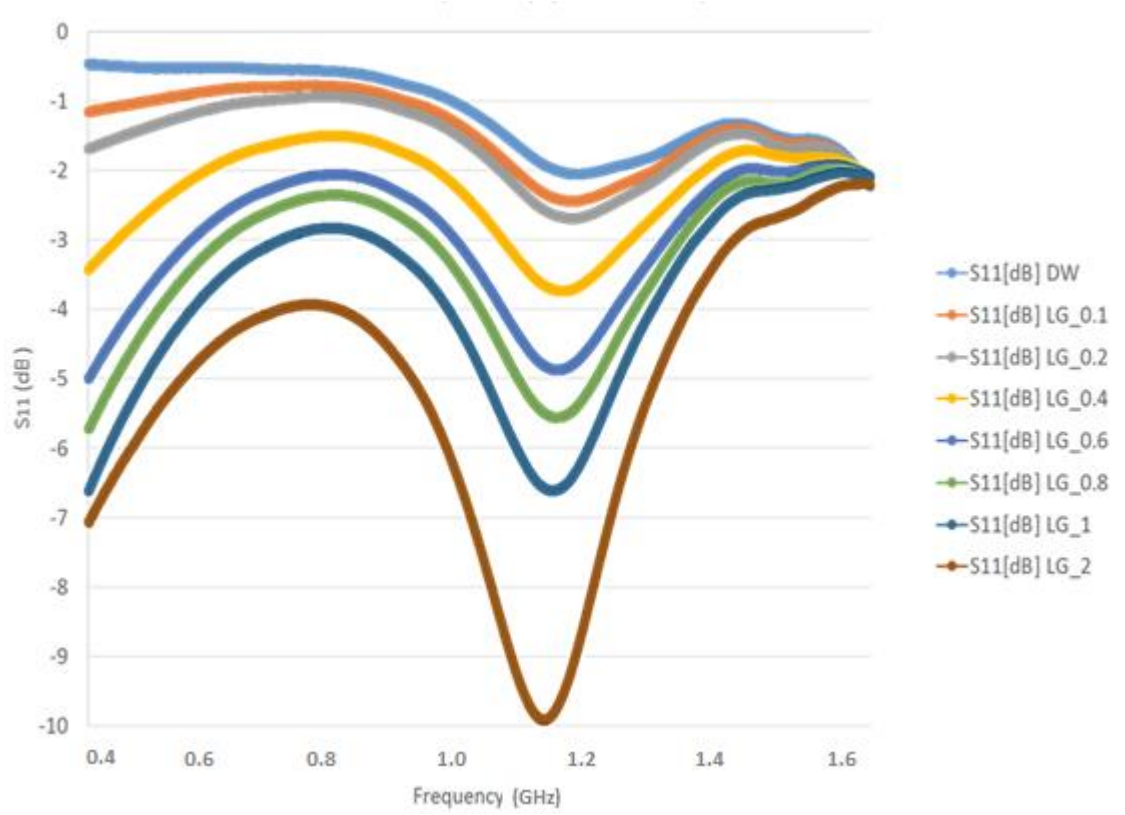


Figure 7.16 S_{11} vs. Frequency Liquid Gold 8-fold samples

7.4.3 Using Sensor C

Figure 7.17 shows raw data from recorded spectral signatures for Acid Descaler via sensor C, whereas, Figure 7.18 distinguishes these varying concentrations at frequency 986 MHz using planar resonator with 94.47 % polynomial correlation.

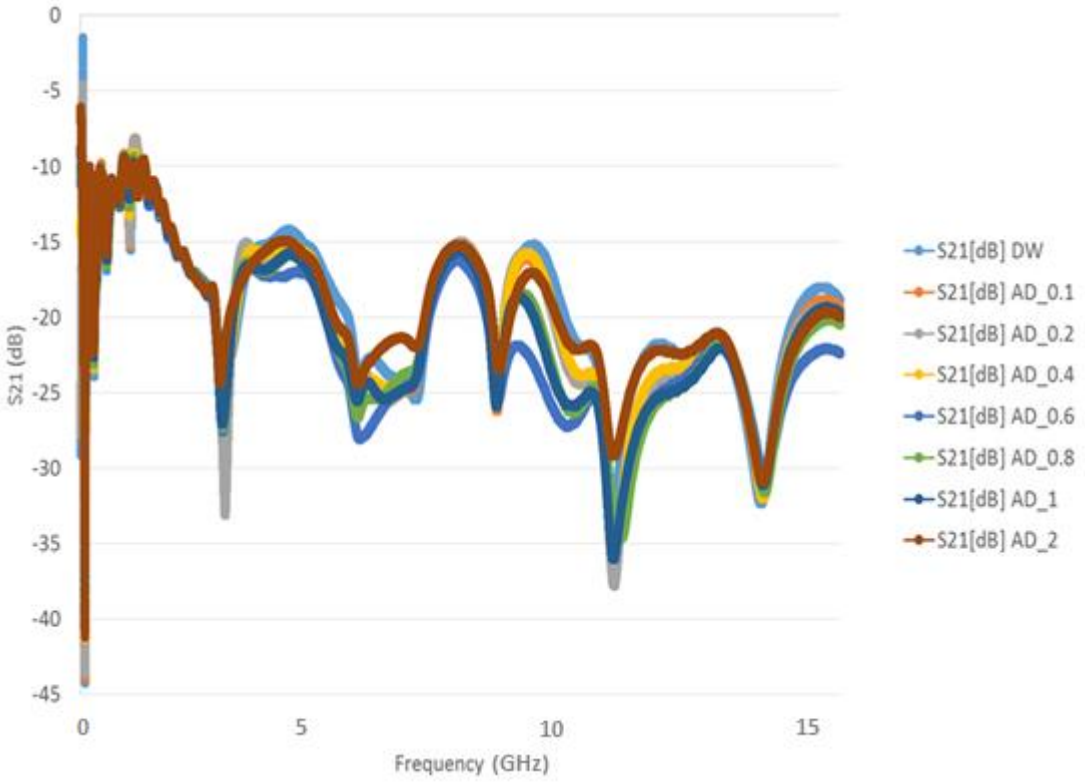


Figure 7.17 S_{21} vs. Frequency Acid Descaler 8-fold samples

Whereas on the other hand Figure 7.19 and Figure 7.20 show similar plots for Liquid Gold (LG) respectively. The optimum polynomial correlation achieved for LG was rather at the very low end of frequency as compared to AD at around 10.5 MHz with 96.65 % R^2 value.

These were the best R^2 values achieved, for polynomial fit, from data analysis of recorded spectral signatures over full sweep of frequencies ranging from 10 MHz to 15 GHz.

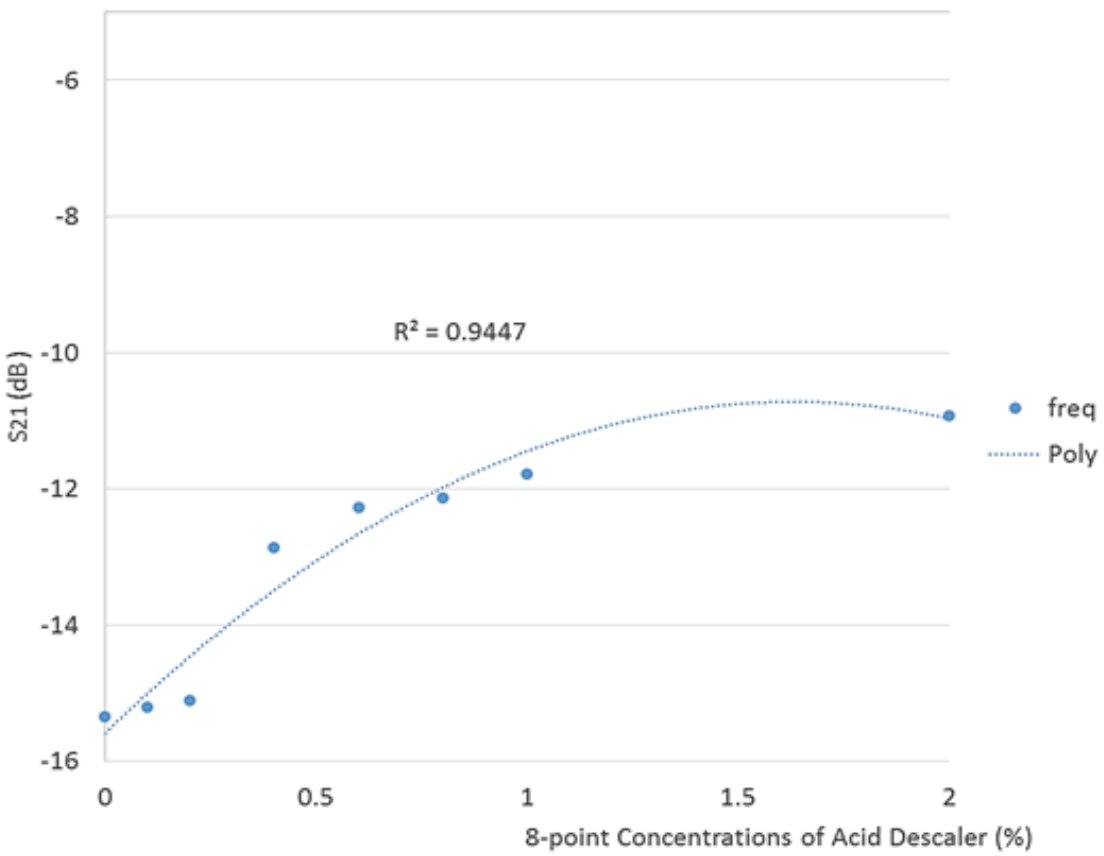


Figure 7.18 S_{21} vs. Concentrations for AD at around 986MHz frequency

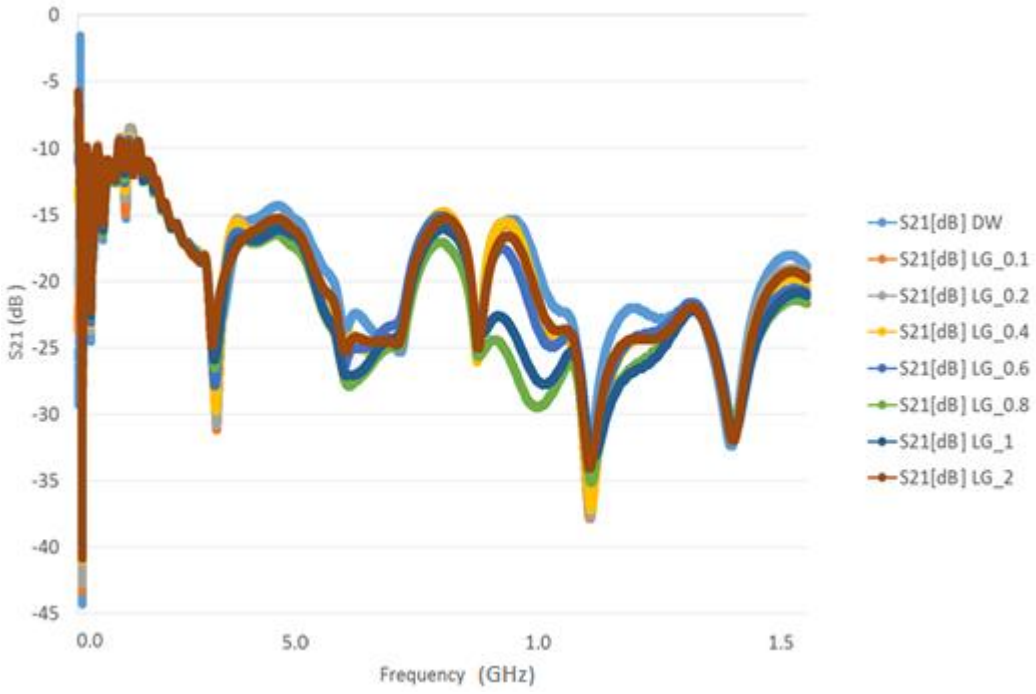


Figure 7.19 S_{21} vs. Frequency Liquid Gold 8-fold samples

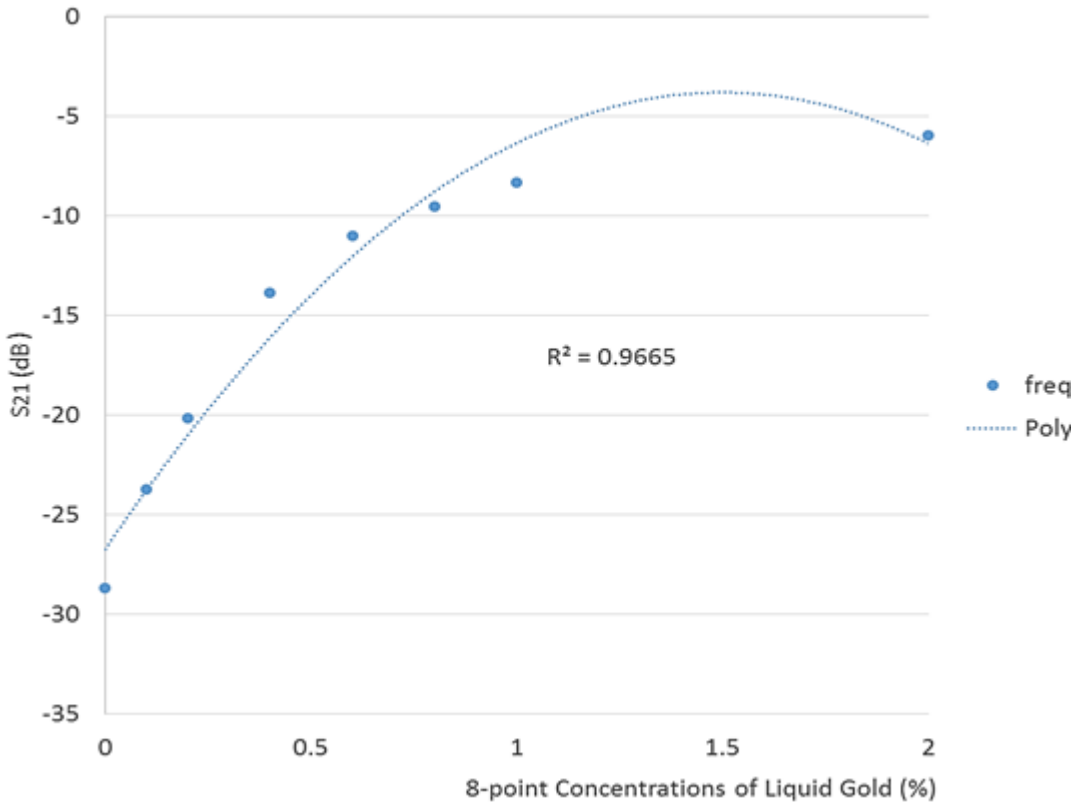


Figure 7.20 S_{21} vs. Concentrations for LG at around 10.5 MHz frequency

7.5 Summary

Urea adulteration up to 1400 ppm, which is double the range of the naturally found limit of urea in milk, was identified and samples were graded based on concentration of urea. The results achieved here demonstrate that the proposed novel technique, using an electromagnetic wave sensor has shown promising results with 96.18% of linear correlation and has the potential to replace the robust existing methodologies, which are tedious due to requirement of reagents preparation, complex as well as time sensitive as well as being costlier than the proposed technique overall.

This method can aid as a non-invasive and real-time inspection method to determine the urea adulteration in milk as it measures the milk samples directly, eliminating several redundant stages of the conventional methods that cost time and resources. It can also be easily incorporated in the existing set-up of milk quality testing and control to overcome the limitation of currently existing technology.

The detergents used as cleansing agents for milk containers were also tested and their varying concentrations detected for both alkali as well as acidic detergent. All three types of sensor were used to understand the detection patterns and behavioural changes of recorded spectra.

CHAPTER 8

COMPARATIVE REVIEW, FINDINGS AND RECOMMENDATIONS

This chapter reviews the entire research project on a comparative analytical basis. The findings made during this research are discussed concerning the current state of the art, in industry as well as in research domains, and relative position of the proposed research is outlined. A thorough discussion is presented to recommend suitable sensing platform based on the comparison of all three types of microwave sensor used in the project, and tested with commercially purchased cow milk (skimmed milk, semi-skimmed milk and whole milk). The following sections reflect on the bespoke milk quality control paradigm for processed (i.e. pasteurised, homogenised and standardised) cow milk sold in commercial markets.

8.1 Review of the Research Project

Figure 8.1 illustrates the revised milk processing and production flow-chart, outlying the current procedural sequence (see Figure 2.1) of the dairy industry, from farms to the consumer with the addition of microwave sensing technology. It is important to note that the inclusion of the proposed microwave sensing technology allows for a testing mechanism that can be performed outside of the laboratory and can be retrofitted to the current industrial process.

The microwave sensors allow the flexibility of a sensing system that is portable, low profile, non-destructive, non-invasive, rapid and reliable. These characteristics allow it to operate in a less restricted environment, such as industrial setup, without the explicit need of technically skilled staff, as in many cases of the existing technologies discussed earlier in this thesis. The biggest advantage of such a system is that there is no need to execute the operations like preparing stock solutions, the addition of reagents to samples under test, mixing and centrifuging and hence the destruction of the samples due to the invasive approach, as in most other standard practices.

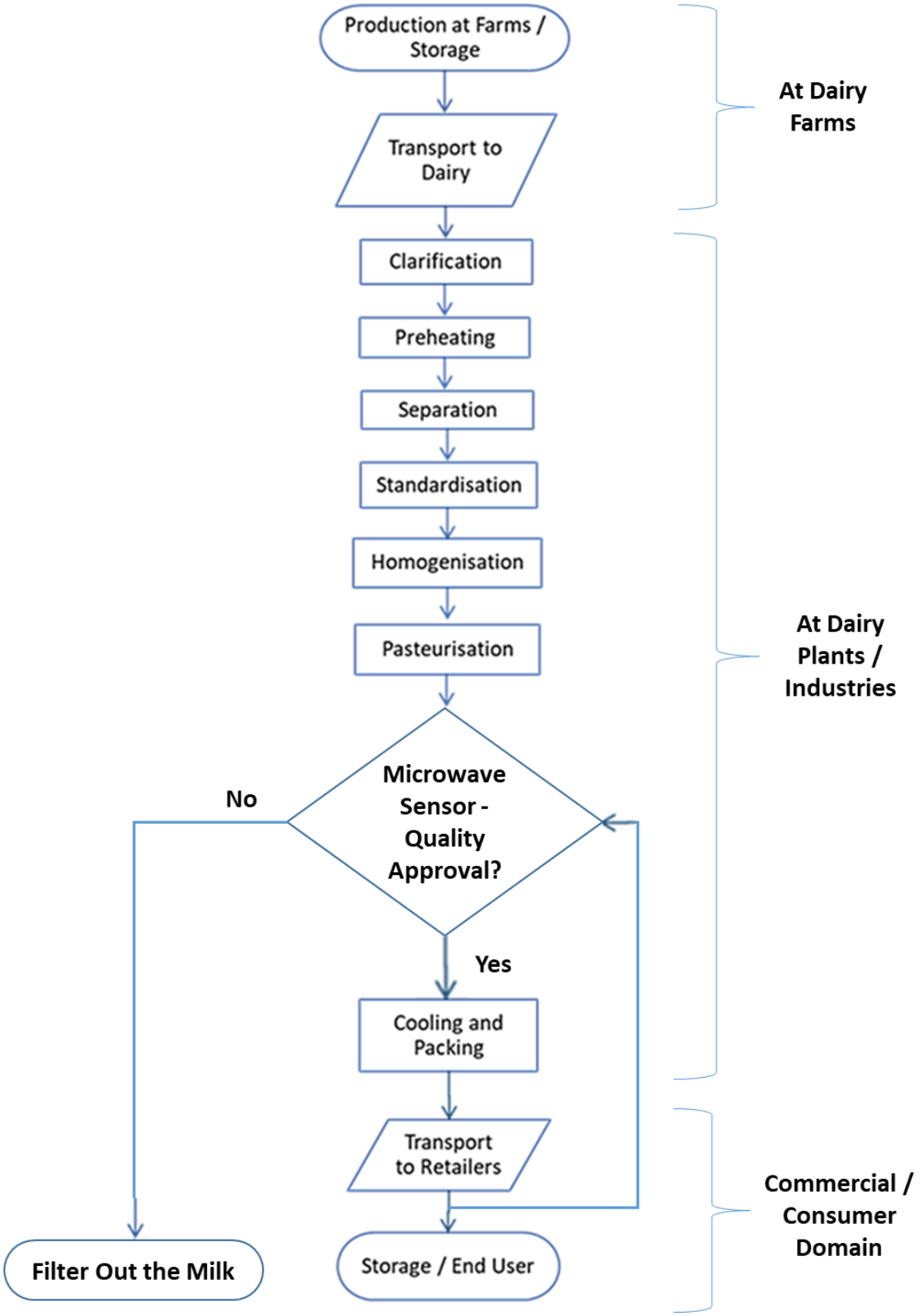
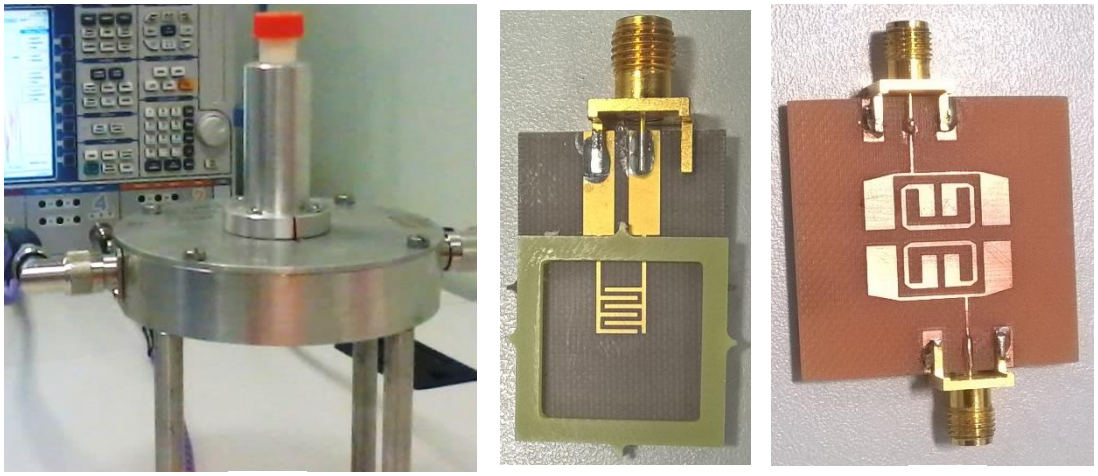


Figure 8.1 Flow-chart of Milk Processing with retrofitted Microwave Sensor System

The experimental results, using microwave spectroscopy, could effectively distinguish between the spoiled milk samples and fresh milk samples, although the spoilage resulting from specific bacteria and detection of presence of particular bacterium was beyond the scope of this project.

Similarly, the composition check was mainly focused at the milk type classification based on the fat content values, and a proof-of concept set of experiments were also carried out for determining protein levels. For the adulteration tests, the use of primarily reported adulterants were used – with urea in milk and detergent in deionised water. The application of these microwave sensors can be further extended to accommodate more adulterants as well as for the detection of a specific bacterium type in the future.



(a) (b) (c)

Figure 8.2 Microwave sensors used in this research: (a) Sensor A, (b) Sensor B, and (c) Sensor C

Figure 8.2 depicts all three sensor types used in this research project. Although these three sensor types seem to be radically different in their design, the common factor associating these microwave devices is the near-field sensing, as discussed earlier in Chapter 3.

The next section discusses the findings of the research project and recommendations based on the interpretations made.

8.2 Findings and Recommendations

The following is the comparison and discussion for each of these three sensor types based on the results from the milk quality testing in a simulated environment, and the actual milk data from experimental measurements. The discussion is made in terms of how the simulation results transpire into real-time experimental data and the affecting factors. The research findings with the help of result analysis are explained on a comparative basis to interpret the outcome in each case.

Figure 8.3 compares the milk test results using sensor A under a simulated environment (HFSS) and in real-world experiments. The reflection coefficient is measured in dB using a Rohde & Schwarz ZVA24 vector network analyser and plotted against frequency. In both the cases, a separation of graphs is seen at around 5 GHz, 5.85 GHz to 6 GHz, and near 6.65 GHz. The results show amplitude shifts at various frequencies, giving the microwave sensing system ability to classify milk types.

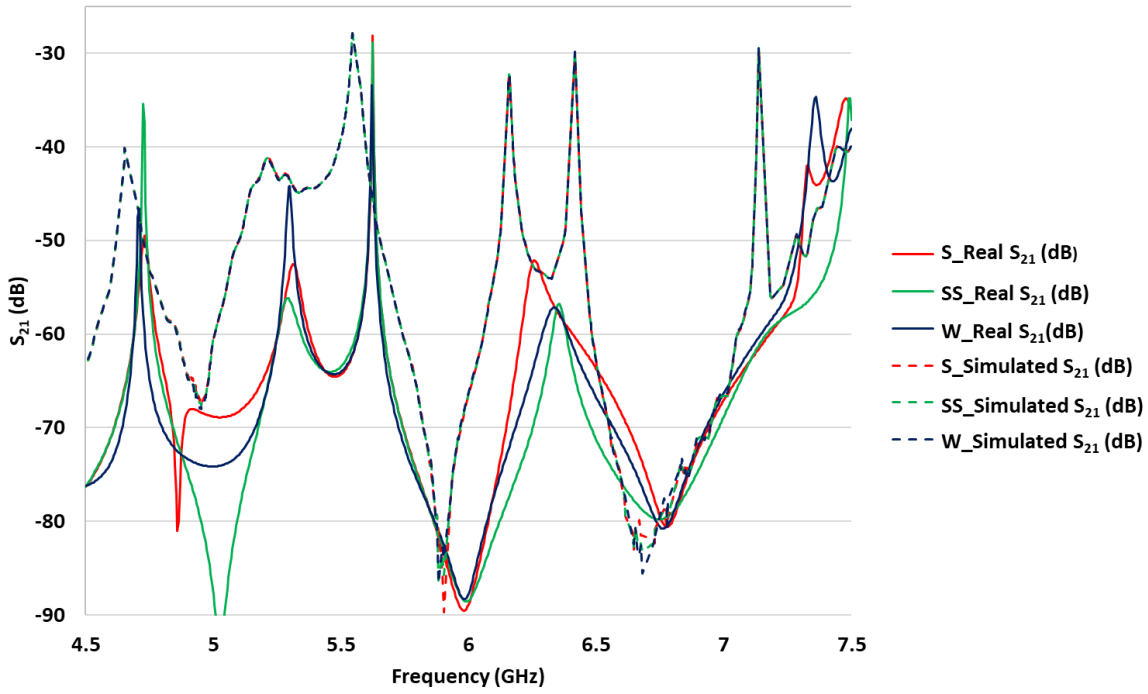


Figure 8.3 Graph of S_{21} vs. frequencies for Skimmed, Semi-skimmed and Whole milk using Sensor A under Simulation and Real-life

Figure 8.4 compares the milk test results using sensor B for simulated and in real-world experiment. This type of sensor primarily gives frequency shifts rather than just the amplitude shifts, between 5.45 GHz and 5.55 GHz in reflection coefficient measurements at the single port-1, which was the case in sensor A as discussed earlier. The sensing system therefore clearly classifies the three milk types through both frequency and amplitude shifts.

Other minor shifts were seen at the frequency 2.45 GHz .

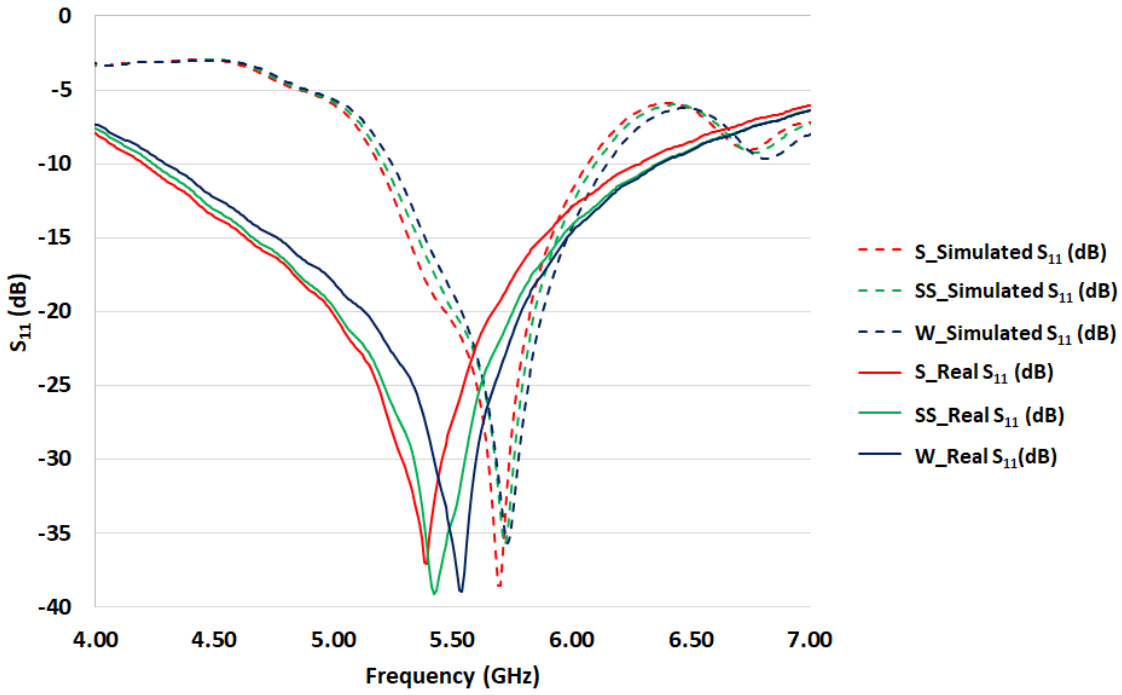


Figure 8.4 Graph of S_{21} vs. frequencies for Skimmed, Semi-skimmed and Whole milk using Sensor B under Simulation and Real-life

Figure 8.5, compares the milk test results for simulated and real experiments using sensor C for skimmed, semi-skimmed, and whole milk. This sensor also shows primarily amplitude shifts with some minor frequency shifts enabling the sensing system to distinguish between the milk samples based on their fat content, at around 1.8 GHz and 6.8 GHz.

The variation between the two results are due to several factors, including the application of mask layer, to protect the sensor, at the time of its fabrication.

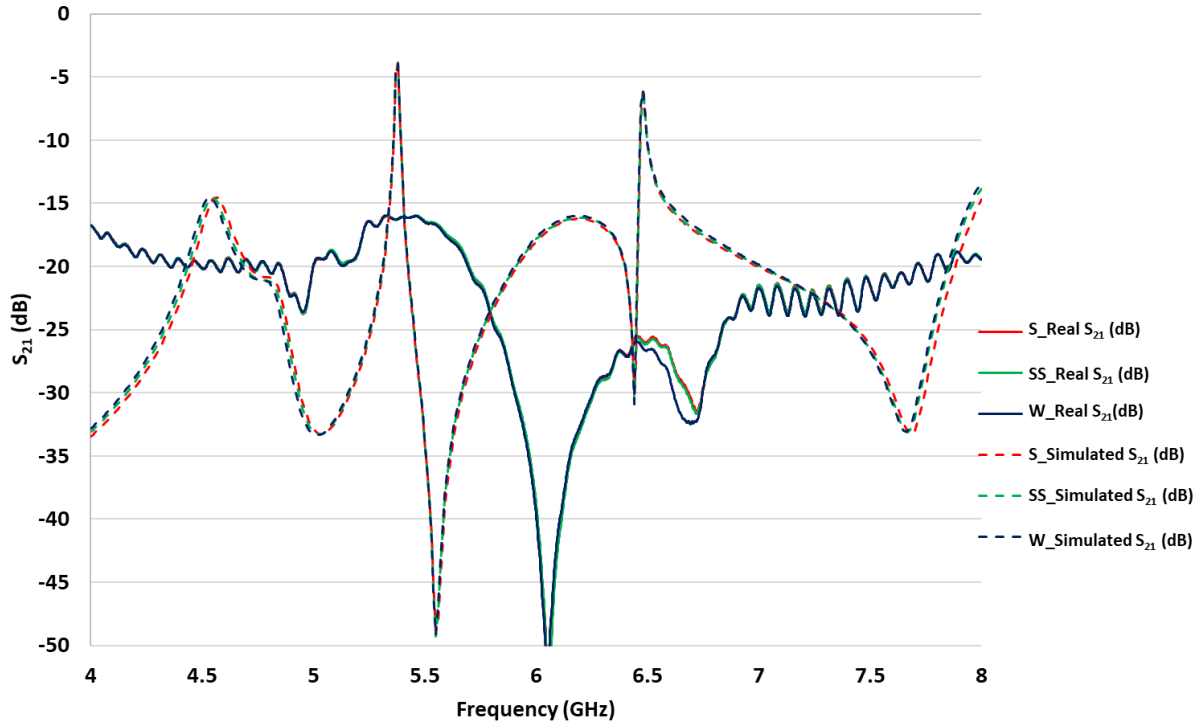


Figure 8.5 Graph of S21 vs. frequencies for Skimmed, Semi-skimmed and Whole milk using Sensor C under Simulation and Real-life

All three sensors show expected results at the resonance frequencies they are designed for, as well as at certain other harmonics; the scattering parameter increases from skimmed, through semi-skimmed to whole milk. The choice of sensor is determined based on the application environment, the size requirement and portability, as well as the size of the sample and the nature of the tests, i.e. invasive or non-invasive.

Table 8.1 compares the three different microwave sensor types used in this research with their primary characteristics. This shows how the sensor parameters evolved, in this project, from one requirement to another. The relationship between response from the individual sensors and the milk parameters were compared to determine the most suitable sensor for each application namely, classification, adulteration and spoilage. Sensor A demonstrates the strongest linear relationship for classification and spoilage whereas Sensor B presents the strongest polynomial fit for adulteration.

All three sensors demonstrate strong correlation and confirm that electromagnetic waves can be used as a potential method to determine milk quality.

Table 8.1 The three microwave sensor types

Sensor	Overall Sensor Dimensions	Type of Material	No. of Ports	Sampling Method	Classifi-cation	Adulte-ration	Spoilage
Sensor A	≈ 130mm x 60mm	Aluminium	2	Test Tube (15 ml)	R^2= 0.98 (Linear Fit)	R^2= 0.97 (Polynomial Fit)	R^2= 0.96 Skimmed R^2= 0.99 Semi-Skimmed R^2= 0.99 Whole (Linear Fit)
Sensor B	≈ 40mm x 20mm	FR4 epoxy +Brass +Gold	1	Pipette (0.5 ml)	R^2= 0.93 (Linear Fit)	R^2= 0.99 (Polynomial Fit)	R^2= 0.92 Skimmed R^2= 0.95 Semi-Skimmed R^2= 0.67 Whole (Linear Fit)
Sensor C	≈ 35mm x 25mm	FR4 epoxy + Copper	2	Fluidic Tube (~ 0.25 ml)	R^2= 0.97 (Linear Fit)	R^2= 0.97 (Polynomial Fit)	R^2= 0.86 Skimmed R^2= 0.89 Semi-Skimmed R^2= 0.79 Whole (Linear Fit)

Sensor A allows for a larger sample volume compared to other two types of sensors, and it can be retrofitted into industrial configuration for real-time continuous monitoring of milk quality.

Sensor B is the smallest of the three but it is invasive in nature, as the milk sample is required to be placed on its surface to enable quality measurements.

Sensor C allowed a fluidic sensor system where milk samples were passed through a polyethylene tube using a peristatic pump. This could be particularly useful in the applications where portable, rapid and non-invasive measurements are primary requirements, e.g. for food inspectors on a routine visit to a milk production and/or storage facility.

8.3 Summary

In this chapter, the proposed sensing technology was mapped with the existing industrial domain as well as research domain practices. It was compared with the existing standards in terms of advantages and limitations including important design constraints such as portability, non-invasive, non-destructive application. It was understood upon experimental results and analysis that the proposed methodology is also non-ionising and less expensive compared to existing standard IR/NIR spectroscopy because of lower frequency range.

In this research work, it was effectively shown that the microwave sensing technology eliminates, or minimises, the limitations of the current state of the art in research as well as in the industrial domain. The biggest advantage of this technology is its low-cost of operation, simple installation with a provision of portability and most importantly, real-time measurements. The proposed technology is employed to detect spoilage of milk over the period of time, determine the type of milk based on its fat contents and is also successfully applied to detect the presence of adulteration.

The following chapter provides concluding remarks and a discussion on further work on the proposed technique.

CHAPTER 9

CONCLUSIONS AND FURTHER WORK

This chapter gives concluding remarks with a brief discussion on the scope for further work for this research project.

9.1 Concluding Remarks

The research project started with a thorough literature study and reviewing current methods in dairy industry as well as research domain for milk quality monitoring and control, with their ongoing challenges. From the study of limitations of current state of the art, it was derived that it has a scope for further improvement where no easy-to-use yet effective technique exists which is also time as well as resource saving after being incorporated.

As explained earlier in Chapter 1 and Chapter 2 followed by review in Chapter 8, it is evident that the industrial setup for the processing of raw milk is robust enough to address the need of milk quality control. This allows the existing mechanism in place to ensure that the acceptable standards of quality are maintained while packaging, of processed milk products, is carried out at dairy plants. Therefore, the aim of this research was particularly targeted towards developing a new milk sensing technology that is suitable beyond the existing industrial robust setup and usable outside the laboratory environment enabling real-time measurements, and at the same time the one that is easily amendable to the setup currently in place.

The preliminary work started with the investigation of the EM wave theory and understanding various types of microwave sensors used in different applications. This investigation lead to selection of three types of microwave sensors namely, cavity resonator, Interdigitated Electrodes (IDE) sensor and Hairpin Sensor as a potential sensing platform for determination of milk quality.

The dielectric property experiments were carried out to evaluate the dielectric characteristics of the three widely sold commercial milk types in the consumer market namely, skimmed milk, semi-skimmed milk, and whole milk using open

ended coaxial technique. Using the obtained results, the theoretical model of three sensors were designed and simulated using High Frequency Structure Simulator (HFSS) for classification, adulteration and spoilage detection.

The 2-port Sensor A, designed and developed at LJMU, was first applied for preliminary tests, as a microwave sensor to classify among different milk types based on their fat content and to determine the spoilage in milk samples occurring over time. For all the tests, the milk bottles were freshly purchased with the longest available span before the expiry date labelled on their packaging, which gave about 7 days of time period by the time best before date were due. The results of these tests were then plotted, data analysed and compared to the theoretical simulation model results as well as the expected outcome.

The primary feature of the resonator sensor A was its characteristics of giving amplitude shifts as the type of milk changes. The deionised water had the lowest value of scattering parameter (i.e. the highest in negative values) and it increased as we go along from lower fat to higher fat content milk types, e.g. from skimmed milk, to the whole milk through semi-skimmed milk. In addition, the milk samples that were spoiled also gave decremented result in scattering parameter vs. frequency plots as compared to the fresh ones, due to the separation of milk solids from water content as the milk spoils. This enables available specifically for liquid samples, both in near-field material sensing applications, namely microwave resonator sensor A and sensor B, were identified and employed in simulations and then in real-world measurements.

Following the proof of concept using both sensors for spoilage detection and milk type classification, a sensor C design based on planar resonator technology, known as hairpin resonator, was designed, simulated, and fabricated for real-world measurements. The results achieved by fabricated sensor were compared with simulation results and also for the case of adulteration of urea in milk, the gold standard methodology involving spectrophotometry was replicated in the laboratories and results with proposed microwave spectroscopy technique were compared and benchmarked followed by measurements of detergents in water as cleaning agents.

Application of a microwave cavity, acting as a resonating sensor device to detect spoilage and the type of milk. A wideband source applied to the cavity helped locate the values of frequency thereby allowing customization of the design that gives optimum results. Once having located the responding frequency values, planar microwave sensors - Interdigitated Electrodes (IDE) were employed to study the invasive milk quality testing and effect of small sample values as low as 0.5ml. Although the IDE sensors are invasive in nature, the characteristics were studied to understand the scope of a non-invasive fluidic sensor that can test milk samples non-destructively and non-invasively.

Finally, the sensor C was designed and developed as a fluidic sensor to achieve more compactness, portability, and non-invasive automation in the testing system. The three objectives of the main aim of project were effectively demonstrated with the optimised design to operate on the same corresponding resonant frequencies for a rapid milk quality testing and monitoring system.

Data analysis was carried out among development of proposed sensor platform. It has been found that developed sensors have a good linear agreement with milk parameters under investigation. The strongest linear relationship was shown by sensor A for classification and spoilage of milk. Whereas Sensor B presented the strongest polynomial fit for adulteration. Sensor C has also demonstrated high linear and polynomial agreement with the classification, adulteration and spoilage of milk.

This technique indeed shows a promising future for design and development of a milk quality testing and control system, for processed and packaged milk types, as it has several advantages over the other already existing standard practices. These advantages include less complexity, higher accuracy, time, cost-effectiveness, and a universal approach to milk types and quality detection. This work presents a very good prototype model for a novel, rapid microwave sensing based quality testing system.

9.2 Further Work

In spite of the acknowledged advantages of the proposed microwave sensor to determine milk quality, it still has a scope for further improvement, which include:

- Expanding research by undertaking experimental work on different animal's milk namely, goat, buffalo, camel
- Undertaking real case study in developing countries such as India
- Improving sensitivity of the design sensor prototype
- Optimising sensor platform by developing RF Circuitry to avoid use of high cost and bench top Vector Network Analyser
- Applying further data analysis and implement machine learning algorithms to enable instant verification of the milk quality

Further optimisation, can help the large-scale or medium-scale retailers, who receive the milk from dairies for selling in packaged form after long stages of transport, to help allow the quality assurance and also to ensure that the customers are getting what they are paying for. This work not only will establish a strong base for the laboratory based research, but also must help towards the problem solving that the dairy industries and end consumers face due to either complexity or costliness of existing standard practices for milk quality control.

REFERENCES

Abdullah, B. M. *et al.* (2014) ‘A Novel Method for Monitoring Structural Metallic Materials Using Microwave NDT’, in *Sensing Technology: Current Status and Future Trends I*. Springer International Publishing. doi: https://doi.org/10.1007/978-3-319-02318-2_9.

Afzal, A. *et al.* (2011) ‘Adulteration and microbiological quality of milk (A review)’, *Pakistan Journal of Nutrition*, 10(12), pp. 1195–1202. doi: 10.3923/pjn.2011.1195.1202.

Al-Holy, M. A. *et al.* (2006) ‘The use of Fourier transform infrared spectroscopy to differentiate Escherichia coli O157:H7 from other bacteria inoculated into apple juice’, *Food Microbiology*, 23(2), pp. 162–168. doi: 10.1016/j.fm.2005.01.017.

Al-Qadiri, H. M. *et al.* (2008) ‘Monitoring quality loss of pasteurized skim milk using visible and short wavelength near-infrared spectroscopy and multivariate analysis.’, *Journal of dairy science*. Elsevier, 91(3), pp. 950–958. doi: 10.3168/jds.2007-0618.

Alcantara, G. P. and Andrade, C. G. M. (2016) ‘A short review of gas sensors based on interdigital electrode’, *2015 IEEE 12th International Conference on Electronic Measurement and Instruments, ICEMI 2015*, 3, pp. 1616–1621. doi: 10.1109/ICEMI.2015.7494489.

Alhajeri, S. H. H. (2010) *Online Electromagnetic Wave Monitoring System for Petroleum Industry Applications*. Liverpool John Moores University.

Anand, M. J. and Sridhar, V. (2018) ‘Characterisation of Gas Sensor Array for Milk Spoilage and Diseases Detection’, 7(2), pp. 48–52.

Atherton, H. V. and Newlander, J. A. (1977) ‘Chemistry and testing of dairy products.’, *Chemistry and testing of dairy products.* AVI Publishing Co. Inc., (Ed. 4). Available at: https://www.cabdirect.org/cabdirect/abstract/19780443856 (Accessed: 2 June 2018).

Azad, T. and Ahmed, S. (2016) ‘Common milk adulteration and their detection techniques’, *International Journal of Food Contamination*. International Journal of Food Contamination, 3(1), p. 22. doi: 10.1186/s40550-016-0045-3.

Bao, X. *et al.* (2018) ‘A Planar One-Port Microwave Microfluidic Sensor for Microliter Liquids Characterization’, *IEEE Journal of Electromagnetics, RF and Microwaves in Medicine and Biology*, 2(1), pp. 1–1. doi: 10.1109/JERM.2018.2807984.

Barbano, D. M. and Lynch, J. M. (2006) ‘Major advances in testing of dairy products: milk component and dairy product attribute testing.’, *Journal of dairy science*. Elsevier, 89(4), pp. 1189–1194. doi: 10.3168/jds.S0022-0302(06)72188-9.

Bhandare, S. G. and Waskar, V. S. (2010) ‘Food safety management for Indian Dairy Industry’, *Indian Dairyman*, 62(6), pp. 42–46. Available at: https://www.cabdirect.org/cabdirect/abstract/20103206895.

Bhandari, V. and Singh, H. (2002) ‘Physical Methods’, *Encyclopedia of Dairy Sciences*. Second. Elsevier Ltd. Available at: https://archive.org/details/EncyclopediaOfDairySciences.

Bhatt, S. R., Singh, A. and Bhatt, S. M. (2008) ‘Assessment of synthetic milk exposure to children of selected population in Uttar Pradesh , India’, *Ind. J. Res. Anvikshiti*, 7, pp. 22–34. Available at: https://www.researchgate.net/publication/266502328.

Bhavadasan, M. K., Rajput, Y. S. and Ganguli, N. G. (1982) ‘A simple colorimetric method for the determination of urea in milk’, *Indian J. Dairy Science*, 33(3), pp. 263–266. Available at: http://krishikosh.egranth.ac.in/bitstream/1/47831/1/dc10.pdf.

Blakey, R. *et al.* (2012) ‘Real-time microwave based sensing method for vegetable oil type verification’, *Procedia Engineering*. Elsevier B.V., 47(December), pp. 623–626. doi: 10.1016/j.proeng.2012.09.224.

Borecki, M. *et al.* (2008) ‘Capillaries as the components of photonic sensor micro-systems’, *Measurement Science and Technology*, 19(6), p. 065202. doi:

10.1088/0957-0233/19/6/065202.

Borecki, M. *et al.* (2009) ‘A method for testing the quality of milk using optical capillaries’, *Photonics Letters of Poland*, 1(1), pp. 37–39. Available at: http://photonics.pl/PLP/index.php/letters/article/view/1-13/13.

Brown, J. V, Ranjith, H. M. P. and Prentice, G. A. (1984) ‘Comparative shelf-lives of skimmed, semi-skimmed and whole milks’, *International Journal of Dairy Technology*, 37(4), pp. 2–5. doi: 10.1111/j.1471-0307.1984.tb00519.x.

Cai, Q. *et al.* (2004) ‘A wireless, remote query glucose biosensor based on a pH-sensitive polymer’, *Analytical Chemistry*, 76(14), pp. 4038–4043. doi: 10.1021/ac0498516.

Chandler, R. E., Ng, S. Y. and Hull, R. R. (1990) ‘Bacterial spoilage of specialty pasteurised milk products.’, *CSIRO Food Research Quarterly*, 50, pp. 11–14. Available at: http://www.cabdirect.org/abstracts/19920449976.html.

Chen, Z. N. (2017) ‘Metamaterials-based Antennas : Translation from Physical Concepts to Engineering Technology’, *Forum for Electromagnetic Research Methods and Application Technologies (FERMAT)*, pp. 1–4. Available at: https://www.e-fermat.org/files/forums/153447fde08810.pdf.

Chiacchierini, E. *et al.* (2003) ‘Milk Soluble Whey Proteins: Fast and Precise Determination with Dumas Method’, *Analytical Letters*, 36(11), p. 2473.

Choi, H. *et al.* (2015) ‘Design and In Vitro Interference Test of Microwave Noninvasive Blood Glucose Monitoring Sensor’, *IEEE Transactions on Microwave Theory and Techniques*, 63(10), pp. 3016–3025. doi: 10.1109/TMTT.2015.2472019.

Cifelli, C. J., German, J. B. and O’Donnell, J. A. (2011) ‘Nutritional and Health-Promoting Properties of Dairy Products: Contribution of Dairy Foods to Nutrient Intake’, *Encyclopedia of Dairy Sciences*. Elsevier Ltd. Available at: https://archive.org/details/EncyclopediaOfDairySciences.

Compagnone, D. *et al.* (2015) ‘Quartz crystal microbalance gas sensor arrays for the quality control of chocolate’, *Sensors and Actuators, B: Chemical*. Elsevier B.V., 207(PB), pp. 1114–1120. doi: 10.1016/j.snb.2014.10.049.

Dai, X. *et al.* (2010) 'Accurate analysis of urea in milk and milk powder by isotope dilution gas chromatography-mass spectrometry', *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. Elsevier B.V., 878(19), pp. 1634–1638. doi: 10.1016/j.jchromb.2010.04.005.

Das, S., Goswami, B. and Biswas, K. (2016) 'Milk Adulteration and Detection: A Review', *Sensor Letters*, 14(1), pp. 4–18. doi: 10.1166/sl.2016.3580.

Deeth, H. C. *et al.* (2002) 'Spoilage patterns of skim and whole milks.', *The Journal of dairy research*, 69(2), pp. 227–241. doi: 10.1017/S0022029901005301.

Dress, P. *et al.* (1998) 'Water-core waveguide for pollution measurements in the deep ultraviolet', *Applied Optics*, 37(21), pp. 4991–4997.

Dunsmore, D. G. (1983) 'The incidence and implications of residues of detergents and sanitizers in dairy products', in Gunther, F. A. (ed.) *RESIDUE REVIEWS: Residues of Pesticides and Other Contaminants in the Total Enviromnent*. doi: 10.1007/978-1-4612-5473-7.

Eat By Date (2018) *The Shelf Life of Milk: How Long Does Milk Last?*, *EatByDate LLC.*

Ellis, D. I. *et al.* (2002) 'Rapid and quantitative detection of the microbial spoilage of meat by fourier transform infrared spectroscopy and machine learning.', *Applied and environmental microbiology*, 68(6), pp. 2822–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12039738 (Accessed: 2 June 2018).

Emerson, D. T. (2002) 'The work of Jagadis Chandra Bose: 100 years of mm-wave research', in *1997 IEEE MTT-S International Microwave Symposium Digest*. Denver: IEEE, pp. 553–556. doi: 10.1109/MWSYM.1997.602853.

FAO (2015a) *Milk and milk products*, *FAO Publications*. Available at: http://www.fao.org/agriculture/dairy-gateway/milk-and-milk-products/en/#.Vc_BJpXH_mQ (Accessed: 1 April 2016).

FAO (2015b) *Milk processing*, *FAO Publications*. Available at: http://www.fao.org/agriculture/dairy-gateway/milk-processing/en/#.Vc-

_vpXH_mQ (Accessed: 1 April 2016).

FAO (2015c) *Milk production*, *FAO Publications*. Available at: http://www.fao.org/agriculture/dairy-gateway/milk-production/en/#.VWvSw89Vikp (Accessed: 1 April 2016).

Fellows, P. and Hampton, A. (1992) *Small-scale food processing - A guide for appropriate equipment*. London: FAO, Intermediate Technology Publications. Available at: http://www.fao.org/wairdocs/x5434e/x5434e00.htm#Contents.

Fok, M. *et al.* (2015) 'A Novel Microwave Sensor to Detect Specific Biomarkers in Human Cerebrospinal Fluid and Their Relationship to Cellular Ischemia During Thoracoabdominal Aortic Aneurysm Repair', *Journal of Medical Systems*, 39(4). doi: 10.1007/s10916-015-0208-4.

Food Safety and Standards Authority of India (2014) *Adulteration in Milk and Milk Products*, *FSSAI*. Available at: http://www.fssai.gov.in/Portals/0/Pdf/Adulteration(26.02.14).pdf (Accessed: 3 April 2016).

Food Standards Agency (2016) *MILK Hygiene on the Dairy Farm A Practical Guide for Milk Producers*. England and Wales. Available at: https://www.food.gov.uk/sites/default/files/multimedia/pdfs/milk-hygiene-guide-for-milk-producers.pdf (Accessed: 10 March 2018).

Fox, P. F. (2011a) 'Introduction | History of Dairy Chemistry', *Encyclopedia of Dairy Sciences*. Second. Elsevier. doi: 10.1016/B978-0-12-374407-4.00542-2.

Fox, P. F. (2011b) 'MILK | Introduction', *Encyclopedia of Dairy Sciences*. Second. Edited by J. W. Fuquay, P. F. Fox, and P. L. H. McSweeney. Elsevier. doi: 10.1016/B978-0-12-374407-4.00310-1.

Francis, P. S., Lewis, S. W. and Lim, K. F. (2002) 'Analytical methodology for the determination of urea: Current practice and future trends', *TrAC - Trends in Analytical Chemistry*, 21(5), pp. 389–400. doi: 10.1016/S0165-9936(02)00507-1.

Fromm, H. I. and Boor, K. J. (2004) 'Characterization of Pasteurized Fluid Milk Shelf-life Attributes', *JOURNAL OF FOOD SCIENCE*. 29th septe, 69(8), pp.

207–214. doi: 10.1111/j.1365-2621.2004.tb09889.x.

FSSAI (2012) ‘Manual of methods of analysis of food: milk and milk products’. Food Safety and Standards Authority of India, Ministry of Health and Family Welfare, Government of India. Available at: http://old.fssai.gov.in/Portals/0/Pdf/Draft_Manuals/MILK_AND_MILK_PRODUCTS.pdf.

Gerosa, S. and Skoet, J. (2013) ‘Milk availability: Current production and demand and medium-term outlook’, in Muehlhoff, E., Bennett, A., and McMahon, D. (eds) *Milk and dairy products in human nutrition*. Rome: FAO Publications, pp. 11–40. Available at: http://www.fao.org/docrep/018/i3396e/i3396e.pdf.

Goh, J. H. *et al.* (2011) ‘Lactate detection using a microwave cavity sensor for biomedical applications’, *2011 Fifth International Conference on Sensing Technology*, pp. 436–441. doi: 10.1109/ICSensT.2011.6137016.

Golding, N. S. (1952) ‘RESAZURN TEST FOR MILK’. United States: United States Patent Office.

Guntupalli, R. *et al.* (2007) ‘Rapid and sensitive magnetoelastic biosensors for the detection of Salmonella typhimurium in a mixed microbial population’, *Journal of Microbiological Methods*, 70(1), pp. 112–118. doi: 10.1016/j.mimet.2007.04.001.

Guo, W. *et al.* (2010) ‘Effects of milk concentration and freshness on microwave dielectric properties’, *Journal of Food Engineering*, 99(3), pp. 344–350. doi: 10.1016/j.jfoodeng.2010.03.015.

Handford, C. E., Campbell, K. and Elliott, C. T. (2016) ‘Impacts of Milk Fraud on Food Safety and Nutrition with Special Emphasis on Developing Countries’, *Comprehensive Reviews in Food Science and Food Safety*, 15(1), pp. 130–142. doi: 10.1111/1541-4337.12181.

Hantsis-Zacharov, E. and Halpern, M. (2007) ‘Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits’, *Applied and Environmental Microbiology*, 73(22), pp. 7162–7168. doi:

10.1128/AEM.00866-07.

Haugen, J. E. *et al.* (2006) 'Application of gas-sensor array technology for detection and monitoring of growth of spoilage bacteria in milk: A model study', *Analytica Chimica Acta*, 565(1), pp. 10–16. doi: 10.1016/j.aca.2006.02.016.

Helland, M. H., Wicklund, T. and Narvhus, J. a. (2004) 'Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings', *International Dairy Journal*, 14(11), pp. 957–965. doi: 10.1016/j.idairyj.2004.03.008.

Hickernell, F. S. (1998) 'Surface acoustic wave devices: a rewarding past, a significant present, and a promising future', *Microwaves and Radar, 1998. MIKON '98., 12th International Conference on*, 4, pp. 159–168 vol.4. doi: 10.1109/MIKON.1998.738468.

Hong, J. S. and Lancaster, M. J. (1998) 'Cross-coupled microstrip hairpin-resonator filters', *IEEE Transactions on Microwave Theory and Techniques*, 46(1), pp. 118–122. doi: 10.1109/22.654931.

Houston, J. (1955) 'A New Method of Butterfat Testing', *International Journal of Dairy Technology*. Wiley/Blackwell (10.1111), 8(1), pp. 47–51. doi: 10.1111/j.1471-0307.1955.tb02694.x.

Huang, P.-C., Liu, C.-F. and Chen, R.-Q. (2017) 'A microwave resonator biosensors for biological cell trapping in portable biomedical application', in *2017 IEEE International Conference on Consumer Electronics - Taiwan (ICCE-TW)*. IEEE, pp. 159–160. doi: 10.1109/ICCE-China.2017.7991044.

Huang, S. *et al.* (2008) 'A remote-query sensor for predictive indication of milk spoilage', *Biosensors and Bioelectronics*, 23(11), pp. 1745–1748. doi: 10.1016/j.bios.2008.01.036.

International Telecommunication Union (2016) *Radio Regulations*. Edition of, *ITU*. Edition of. Geneva, Switzerland: International Telecommunication Union. doi: 10.1016/B978-0-12-394583-9.00020-X.

Janzen, J. J., Bishop, J. R. and Bodine, a. B. (1982) 'Relationship of Protease Activity to Shelf-Life of Skim and Whole Milk', *Journal of Dairy Science*, 65(12),

pp. 2237–2240. doi: 10.3168/jds.S0022-0302(82)82492-2.

Joshi, K. H. *et al.* (2015) ‘Online monitoring of milk quality using electromagnetic wave sensors’, in *2015 9th International Conference on Sensing Technology (ICST)*. Auckland: IEEE, pp. 700–705. doi: 10.1109/ICSensT.2015.7438487.

Joshi, K. H. *et al.* (2017) ‘Milk Quality Monitoring Using Electromagnetic Wave Sensors’, in Mukhopadhyay, S. C. et al. (eds) *Sensors for Everyday Life: Environmental and Food Engineering*. Smart Sens. Springer International Publishing AG, pp. 121–134. doi: 10.1007/978-3-319-47322-2_10.

Kenny, M. (2013) ‘Safety and quality’, in Muehlhoff, E., Bennett, A., and McMahon, D. (eds) *Milk and dairy products in human nutrition*. Rome: FAO Publications, pp. 243–274. Available at: http://www.fao.org/docrep/018/i3396e/i3396e.pdf.

Klaenhammer, T. R. (1988) ‘Bacteriocins of lactic acid bacteria.’, *Biochimie*, 70(3), pp. 337–349. doi: 10.1016/0300-9084(88)90206-4.

Kleyn, D. H. *et al.* (2001) ‘Determination of Fat in Raw and Processed Milks by the Gerber Method: Collaborative Study’, *Journal of AOAC International*, 84(5), pp. 1499–1508.

Kohlmann, K. L. *et al.* (1991) ‘Production of proteases by psychrotrophic microorganisms.’, *Journal of dairy science*, 74(10), pp. 3275–3283. doi: 10.3168/jds.S0022-0302(91)78513-5.

Korostynska, O., Mason, A. and Al-Shamma’A, A. I. (2012) ‘Proof-of-concept microwave sensor on flexible substrate for real-time water composition analysis’, *Proceedings of the International Conference on Sensing Technology, ICST*, pp. 547–550. doi: 10.1109/ICSensT.2012.6461739.

Lars Nygaard, G., Torben Lapp, B. and Börkur Arnvidarson, N. (1993) ‘Method of determining urea in milk’. Denmark. Available at: https://patents.justia.com/patent/5252829.

Lee, Y. G. *et al.* (2009) ‘A rapid and selective method for monitoring the growth of coliforms in milk using the combination of amperometric sensor and reducing of methylene blue’, *Sensors and Actuators, B: Chemical*, 141(2), pp. 575–580.

doi: 10.1016/j.snb.2009.06.028.

Li, L. L. *et al.* (2018) 'An Interdigital Electrode Probe for Detection, Localization and Evaluation of Surface Notch-Type Damage in Metals', *Sensors*. Multidisciplinary Digital Publishing Institute, 18(2), p. 371. doi: 10.3390/s18020371.

Li, X. and Jiang, Y. (2010) *Design of a Cylindrical Cavity Resonator for Measurements of Electrical Properties of Dielectric Materials*, *radio center Glave university*. Gavle University. Available at: http://urn.kb.se/resolve?urn=urn:nbn:se:hig:diva-7687.

Lima, M. J. R., Fernandes, S. M. V. and Rangel, A. O. S. S. (2004) 'Enzymatic determination of urea in milk by sequential injection with spectrophotometric and conductometric detection', *Journal of Agricultural and Food Chemistry*, 52(23), pp. 6887–6890. doi: 10.1021/jf0488312.

Liu, C.-F. *et al.* (2018) 'Improved hairpin resonator for microfluidic sensing', *Sensors and Materials*, 30(5), pp. 979–990. doi: 10.18494/SAM.2018.1801.

Lu, M. *et al.* (2013) 'Milk Spoilage: Methods and Practices of Detecting Milk Quality', *Food and Nutrition Sciences*, 04(07), pp. 113–123. doi: 10.4236/fns.2013.47A014.

Luzzana, M. and Giardino, R. (1999) 'Urea determination in milk by a differential pH technique', *Lait*, 79(2), pp. 261–267. Available at: https://hal.archives-ouvertes.fr/hal-00929648/document.

Mamishev, A. V. *et al.* (2004) 'Interdigital sensors and transducers', *Proceedings of the IEEE*, 92(5), pp. 808–844. doi: 10.1109/JPROC.2004.826603.

Matheson, A. R. and Otten, P. (1999) *Automation of the Rose Gottlieb method for fat determination in dairy products using an expert system*, *American laboratory*.

Mazlan, N. S. *et al.* (2017) 'Interdigitated electrodes as impedance and capacitance biosensors: A review', *AIP Conference Proceedings*, 1885(October). doi: 10.1063/1.5002470.

Mee Sim, M. Y. *et al.* (2003) 'Monitoring of Milk Quality With Disposable Taste Sensor', *Sensors*. 11th Septe, 3(9), pp. 340–349. doi: 10.3390/s30900340.

Mehdizadeh, M. (2015a) 'Fundamentals of field applicators and probes at radiofrequencies and microwave frequencies', in *Microwave/RF Applicators and Probes for Material Heating, Sensing, and Plasma Generation*. Second. Denver: Elsevier Inc., pp. 34–67. doi: http://dx.doi.org/10.1016/B978-0-323-32256-0.00002-6.

Mehdizadeh, M. (2015b) 'Introduction', in *Microwave/RF Applicators and Probes for Material Heating, Sensing, and Plasma Generation*. Second. Elsevier Inc., pp. xvii–xix. doi: 10.1016/B978-0-323-32256-0.00014-2.

Mehdizadeh, M. (2015c) 'The impact of fields on materials at microwave and radio frequencies', in *Microwave/RF Applicators and Probes for Material Heating, Sensing, and Plasma Generation*. Second. Elsevier Inc., pp. 1–33. doi: 10.1016/B978-0-323-32256-0.00001-4.

Ministry of Agriculture - Republic of Kenya (2011) 'Milk testing and Quality Control'. Naivasha: FAO/TCP/KEN/6611 Project, p. 28. Available at: http://www.fao.org/AG/AGAInfo/resources/documents/MPGuide/mpguide2.htm.

Mohd Bahar, A. A. *et al.* (2017) 'High-efficiency microwave planar resonator sensor based on bridge split ring topology', *IEEE Microwave and Wireless Components Letters*, 27(6), pp. 545–547. doi: 10.1109/LMWC.2017.2701338.

Moore, H. C. and Morse, P. A. (1926) 'A Babcock-Gerber Method for Determining the Percentage of Fat in Ice Cream*', *Journal of Dairy Science*. Elsevier, 9(3), pp. 276–285. doi: 10.3168/jds.S0022-0302(26)93898-8.

Motarjemi, Y. *et al.* (2014) 'Milk and Dairy Products', in *Food Safety Management: A Practical Guide for the Food Industry*, pp. 83–117. doi: 10.1016/B978-0-12-381504-0.00005-6.

Muehlhoff, E., Bennett, A. and McMahon, D. (2013) 'Introduction', in Muehlhoff, E., Bennett, A., and McMahon, D. (eds) *Milk and dairy products in human nutrition*. Rome: FAO Publications.

Muir, D. D. and Sweetsur, A. W. M. (1976) 'The influence of naturally occurring

levels of urea on the heat stability of bulk milk', *Journal of Dairy Research*, 43, pp. 495–499. doi: 10.1017/S0022029900016083.

Murphy, S. C. (2009) *SHELF-LIFE OF MILK PRODUCTS - MICROBIAL SPOILAGE - The Evaluation of Shelf-Life*. Ithaca, New York.

My Agriculture Information Bank (2015) *Milk Testing - Clot on Boiling Test (COB)*, *Practicals on Technology of Milk & Milk Products*. Available at: http://www.agriinfo.in/default.aspx?page=topic&superid=9&topicid=711 (Accessed: 11 March 2018).

Nascimento, C. F. *et al.* (2017) 'Recent advances on determination of milk adulterants', *Food Chemistry*. Elsevier Ltd, 221, pp. 1232–1244. doi: 10.1016/j.foodchem.2016.11.034.

Di Natale, C. *et al.* (2000) 'Electronic nose and electronic tongue integration for improved classification of clinical and food samples', *Sensors and Actuators B: Chemical*, 64(1), pp. 15–21. doi: 10.1016/S0925-4005(99)00477-3.

News Desk (2012) 'Raw Milk Still Suspect in 14 Missouri E. Coli Cases', *Food Safety News*. Available at: http://www.foodsafetynews.com/2012/05/missouri-says-14-e-coli-cases-may-be-from-raw-milk/#.WnRXb7p2u70 (Accessed: 21 February 2018).

News Desk (2015) 'Raw Milk Recalled in California for Campylobacter Contamination', *Food Safety News*. Available at: http://www.foodsafetynews.com/2015/10/raw-milk-recalled-in-california-for-campylobacter-contamination/#.VnJ4MFduTOs (Accessed: 21 February 2018).

Nicolaou-Markide, N. (2011) *The use of analytical techniques for the rapid detection of microbial spoilage and adulteration in milk*, *Faculty of Engineering and Physical Sciences, School of Chemistry*. The University of Manchester. Available at: https://www.escholar.manchester.ac.uk/api/datastream?publicationPid=uk-ac-man-scw:122017&datastreamId=FULL-TEXT.PDF.

Nicolaou, N., Xu, Y. and Goodacre, R. (2010) 'Fourier transform infrared spectroscopy and multivariate analysis for the detection and quantification of

different milk species.', *Journal of dairy science*, 93(12), pp. 5651–5660. doi: 10.3168/jds.2010-3619.

Nikoleli, G. P., Nikolelis, D. P. and Methenitis, C. (2010) 'Construction of a simple optical sensor based on air stable lipid film with incorporated urease for the rapid detection of urea in milk', *Analytica Chimica Acta*. Elsevier B.V., 675(1), pp. 58–63. doi: 10.1016/j.aca.2010.07.014.

NPCS Team (2014) *Market Research Report on Milk Processing & Dairy Products in India (Butter, Yogurt, UHT Milk, Cheese, Ice Cream, Ghee & Other Products)*. NIIR Project Consultancy Services. Available at: http://www.niir.org/books/book/market-research-report-on-milk-processing-dairy-products-in-india-butter-yogurt-uht-milk-cheese-ice-cream-ghee-other-products-npcs-team/isbn-9789381039496/zb,,18b7c,a,72,0,a/index.html.

Nyfors, E. (2000) 'Industrial Microwave Sensors - A Review', *Subsurface Sensing Technologies and Applications*, 1(1), pp. 23–43.

OECD and FAO (eds) (2015) *OECD-FAO Agricultural Outlook 2015-2024*. Paris: OECD Publishing. doi: 10.1787/agr_outlook-2015-en.

Osman, S. B. *et al.* (2014) 'Application of Microwave Spectroscopy Analysis on Determining Quality of Vegetable Oil', in *Proceedings of the 8th International Conference on Sensing Technology*. Liverpool, pp. 556–559. Available at: http://s2is.org/ICST-2014/papers/1569978577.pdf.

Paradkar, M. M., Singhal, R. S. and Kulkarni, P. R. (2000) 'An approach to the detection of synthetic milk in dairy milk : 1. Detection of urea', *International Journal of Dairy Technology*, 53(3), pp. 87–91. doi: 10.1111/j.1471-0307.2000.tb02666.x.

Povolo, M. and Contarini, G. (2009) 'Fast gas chromatography: Applications in milk fat analysis', *Lipid Technology*, 21(4), pp. 88–90. doi: 10.1002/lite.200900018.

Puckett, L. G. *et al.* (2003) 'Monitoring blood coagulation with magnetoelastic sensors', *Biosensors and Bioelectronics*, 18(5–6), pp. 675–681. doi: 10.1016/S0956-5663(03)00033-2.

Reinemann, D. J. and Helgren, J. M. (2004) 'Online Milk Sensing Issues for Automatic Milking', 0300(04).

Renny, E. F. *et al.* (2005) 'Enzyme based sensor for detection of urea in milk', *Biotechnology and Biotechnological Equipment*, 19(2), pp. 198–201. doi: 10.1080/13102818.2005.10817216.

Rivadeneyra, A. *et al.* (2016) 'Printed electrodes structures as capacitive humidity sensors: A comparison', *Sensors and Actuators A: Physical*. Elsevier, 244, pp. 56–65. doi: 10.1016/J.SNA.2016.03.023.

Romaniuk, R. S. and Dorosz, J. (2006) 'Technology of soft-glass optical fiber capillaries', *Photonics Applications in Astronomy, Communications, Industry, and High-Energy Physics Experiments 2006*, (October), p. 634710.1--634710.9. doi: 10.1117/12.714619.

Ruan, C., Zeng, K. and Grimes, C. a. (2003) 'A mass-sensitive pH sensor based on a stimuli-responsive polymer', *Analytica Chimica Acta*, 497(1–2), pp. 123–131. doi: 10.1016/j.aca.2003.08.051.

Sagawa, M., Takahashi, K. and Makimoto, M. (1989) 'Miniaturized Hairpin Resonator Filters And Their Application To Receiver Front-End Mic'S', *IEEE Transactions on Microwave Theory and Techniques*, 37(12), pp. 1991–1997. doi: 10.1109/22.44113.

Santos, M. V *et al.* (2003) 'Sensory threshold of off-flavors caused by proteolysis and lipolysis in milk.', *Journal of dairy science*. Elsevier, 86(5), pp. 1601–1607. doi: 10.3168/jds.S0022-0302(03)73745-X.

Saxena, S. (2016) 'Is the Milk You're Drinking safe? 4 Easy Ways to Spot Adulteration', *NDTV FOOD*, 18 March. Available at: http://food.ndtv.com/food-drinks/is-the-milk-youre-drinking-safe-easy-ways-to-spot-adulteration-1288174.

Sharma, P., Lao, L. and Falcone, G. (2018) 'A Microwave Cavity Resonator Sensor for Water-In-Oil Measurements', *Sensors and Actuators B: Chemical*. Elsevier B.V., 262, pp. 200–210. doi: 10.1016/j.snb.2018.01.211.

Siirtola, T. V. A. (2000) 'Quality Control Manual: Raw Milk and Milk Products'. Apia: FAO: Establishment of Regional Reference Centre for Milk Processing

and Marketing. Available at: http://www.fao.org/ag/againfo/themes/documents/lps/dairy/dap/qcm1.htm (Accessed: 11 March 2018).

Singh, K., Soni, B. K. and Nirmal, A. V. (2018) 'Wideband microstrip band stop filters realization with interdigital topologies', *International Journal of Information Technology*. Springer Singapore. doi: 10.1007/s41870-018-0151-y.

Singuluri, H. and Sukumaran, M. (2014) 'Milk Adulteration in Hyderabad, India – A Comparative Study on the Levels of Different Adulterants Present in Milk', *Journal of Chromatography & Separation Techniques*, 05(01), pp. 1–3. doi: 10.4172/2157-7064.1000212.

Subramanian, A., Prabhakar, V. and Rodriguez-Saona, L. (2011) 'Analytical Methods | Infrared Spectroscopy in Dairy Analysis', *Encyclopedia of Dairy Sciences*. Second. Elsevier. doi: 10.1016/B978-0-12-374407-4.00011-X.

The Dairy Council (2016) 'Milk Factsheet', *The Dairy Council*. London: The Dairy Council. Available at: http://www.milk.co.uk/resources/resource.aspx?intResourceID=153.

The Hans India (2017) 'Milk adulteration racket busted; 11 held', 15 February. Available at: http://www.thehansindia.com/posts/index/Crime/2017-02-15/Milk-adulteration-racket-busted-11-held/280780.

Townes, C. (1952) 'The present status of microwave spectroscopy', *Annals of the New York Academy of Sciences*, 55, pp. 745–750. doi: 10.1111/j.1749-6632.1952.tb26592.x.

Trivedi, U. B. *et al.* (2009) 'Potentiometric biosensor for urea determination in milk', *Sensors and Actuators, B: Chemical*, 140(1), pp. 260–266. doi: 10.1016/j.snb.2009.04.022.

Wahba, W., Abdalla, M. A. and Allam, A. M. M. (2016) 'Experimental verification of a compact zeroth order metamaterial substrate integrated waveguide antenna', *Progress In Electromagnetics Research C*, 67(May), pp. 193–201. doi: 10.2528/PIERC16052906.

Wangler, T. P., Wangler, T. P. and Wiley InterScience (Online service) (2008)

RF Linear Accelerators. 2nd, Compl edn. Wiley. Available at: https://www.wiley.com/en-gb/RF+Linear+Accelerators,+2nd,+Completely+Revised+and+Enlarged+Edition-p-9783527406807 (Accessed: 5 June 2018).

WHO (2009) *Toxicological and Health Aspects of Melamine and Cyanuric Acid*. Ottawa. Available at: http://www.who.int/foodsafety/publications/chem/Melamine_report09.pdf.

WHO (2017) *Food Safety*, *World Health Organisation*. Available at: http://www.who.int/news-room/fact-sheets/detail/food-safety (Accessed: 7 August 2018).

Winquist, F. *et al.* (1998) 'Monitoring of freshness of milk by an electronic tongue on the basis of voltammetry', *Measurement Science and Technology*. 17th Septe, 9(12), pp. 1937–1946. doi: 10.1088/0957-0233/9/12/002.

Yagoub, S. O., Bellow, F. A. and El Zubeir, I. E. M. (2008) 'Effect of Temperature and Storage Period on the Constituents of Milk Inoculated with Pseudomonas aeruginosa', *Research Journal of Microbiology*, 3(1), pp. 30–34.

Zarifi, M. H., Thundat, T. and Daneshmand, M. (2015) 'High resolution microwave microstrip resonator for sensing applications', *Sensors and Actuators A*, 233, pp. 224–230. doi: 10.1016/j.sna.2015.06.031.

Zhu, X. *et al.* (2015) 'Dielectric Properties of Raw Milk as Functions of Protein Content and Temperature', pp. 670–680. doi: 10.1007/s11947-014-1440-5.

APPENDIX A

ABBREVIATIONS AND ACRONYMS

ADSA	American Dairy Science Association
API	Analytical Profile Index
BMSRT	Bridge Multiple Split Ring Resonator Topology
BTB	Bromothymol Blue
C. burnetii	Coxiella burnetii
COB	Clot On Boiling
COSHH	Control of Substances Hazardous to Health (Regulations)
DT	Detection Time
EM	Electromagnetic
FAO	Food and Agriculture Organisation
HUS	Hemolytic Uremic Syndrome
E. coli	Escherichia coli
EU	European Union
FR-4	Reinforced epoxy laminate (Fibre)
FSA	Food Standards Agency
FSSAI	Food Safety and Standards Authority of India
FT-IR	Fourier Transform-Infra Red
GC	Gas Chromatography
GC/IDMS	Gas Chromatography/Isotope Dilution Mass Spectrometry
HTST	High Temperature-Short Time
IDE	Interdigitated Electrodes
IR	Infra-Red
ISM	Industrial, Scientific and Medical
ITU	International Telecommunication Union
L. monocytogenes	Listeria monocytogenes
LASER	Light Amplification by Stimulated Emission of Radiation
LJMU	Liverpool John Moores University
LOD	Limit of Detection
LTLT	Low Temperature-Long Time
M. tuberculosis	Mycobacterium tuberculosis

MOS	Metal Oxide Semiconductor
MOSFET	Metal Oxide Semiconductor Field Effect Transistor
MUT	Material Under Test
NIR	Near Infra-Red
NPCS	NIIR Project Consultancy Services
NPN	Non-Protein Nitrogen
OECD	Organisation for Economic Cooperation and Development
PCA	Principal Component Analysis
PLS	Partial Least Squares
RADAR	Radio Detection And Ranging
RC	Resistance Capacitance
RFM	Radio Frequency and Microwave
S. aureus	Staphylococcus aureus
S. marcescens	Serratia marcescens
SAW	Surface Acoustic Wave
SIMCA	Soft Independent Modelling of Class Analogy
SNF	Solid Non-Fat
SPEAG	Schmid & Partner Engineering AG
SPSS	Statistical Programme for Social Scientist
Ssp. anaerobius	Subspecies anaerobius
SW NIR	Short Wavelength Near Infra-Red
TOSM	Through Open Short Match
TVC	Total Viable Count
UHT	Ultra-High Temperature
US	United States
UV	Ultra Violet
VIS	Visible spectrum
VNA	Vector Network Analyser
WHO	World Health Organisation

APPENDIX B

NOMENCLATURE

Ag	Argentum (Silver)
AgCl	Argentum (Silver) chloride
BH_3O_3	Boric acid
°C	Degree Celsius
Cal	Calorie (unit of energy)
$CO(NH_2)_2$	Urea
dB	Decibel; the ratio of one value of a physical property to another on a logarithmic scale (Power Ratio: $10^{\wedge 1/10}$)
dBm	Decibel-milliwatt; the power ratio in decibels (dB) of the measured power referenced to one milliwatt (mW)
$\bar{\nabla}$	Curl operator (vector operator that describes the infinitesimal rotation of a vector field in three-dimensional Euclidean space) [called: Del or Nabla Symbol]
∇^2	Laplace operator or Laplacian (differential operator given by the divergence of the gradient of a function on Euclidean space)
$\bar{E}$	Electric Field Vector
ε	Complex-valued dielectric permittivity
ε_0	Permittivity in free space ($8.85 \times 10^{-12}\ F/m$)
ε′	Real component of complex-valued dielectric permittivity (Relative permittivity)
ε″	Imaginary component of complex-valued dielectric permittivity (Dielectric loss factor)
F	Farad (SI unit of capacitance: 1 Farad = 1 Coulomb / 1 Volt)
f	frequency (of signal waves)
g	gram (unit of mass)
GHz	Giga Hertz (10^9 Hz)
H	Henry (SI unit of inductance: 1 Henry = 1 Weber / 1 Ampere)
$\bar{H}$	Magnetic Field Vector (*H*: magnetic field strength; Ampere / metre)
H_2O	Water
H_2O_2	Hydrogen Peroxide
Hz	Hertz (unit of frequency)
J	Joule (unit of energy)
$\bar{J}$	Current density (Ampere / metre^2)

j	Complex operator ($\sqrt{-1}$)
k	Wave number ($k = \omega\sqrt{\mu\varepsilon}$)
kcal	Kilo calorie (10^3 Cal)
KHz	Kilo Hertz (10^3 Hz)
kJ	Kilo Joule (10^3 J); 1 kJ = 0.239006 Calorie
m	Metre (unit of length)
mg	Milligram
MHz	Mega Hertz (10^6 Hz)
ml	Millilitre
mm	Millimetre
ms	millisecond
NaCl	Sodium Chloride (Salt)
NH_3	Ammonia
NH_4^+	Ammonium
pH	Hydrogen ion concentration
r / R	Correlation Coefficient
r^2 / R^2	Coefficient of Determination
S_{11}	Scattering Parameter (input port-1 reflection coefficient)
S_{21}	Scattering Parameter (input port-1 to output port-2 transmission coefficient)
$\tan\delta$	Loss Tangent ($\frac{\varepsilon_r''}{\varepsilon_r'}$)
σ	Sigma (Conductance)
μ	Mu (Permeability)
μ_0	Permeability in free space ($4\pi \times 10^{-7}\ H/m$)
λ	Lambda (Wavelength)
ω	Angular frequency ($2\pi f$)
Wb	Weber (SI unit of magnetic flux)

COSHH

COSHH Risk Assessment Form

Name of Faculty Faculty of Engineering and Technology		**Name of School/Dept.** School of Built Environment / BEST Research Institute
Date of Assessment 10th February 2017		**Site/Location/Room No.** Industrial Chemistry Lab, Laboratory 4
Description of Activity/Procedure/Process (include SOPs as a Control) Adulteration of milk with Urea, Use of DMAB, HCL, TCA, Phosphate pH 7.0 Buffer		
Name of Techniques to be Used Sample preparation, Centrifugation, Spectrophotometry		
Name of Assessor/Supervising Assessor Keyur Joshi / Prof. Andy Shaw	**Signature** K. H. Joshi / A. Shaw	**Tel No.** 01512314252 **E-mail** K.H.Joshi@2014.ljmu.ac.uk
Persons at Risk (Staff/Students/Others?) Low risk to student		Total number of people in lab Maximum 5 people
Is Health Surveillance Required? No **Health Surveillance is required if the procedure involves substances which are respiratory sensitisers or skin sensitisers (risk phrases R42, R43 or R42/43). If other substances with potential health effects are used and if any health effects are observed that is believed to have resulted from its use then Occupational Health should be contacted. Consideration should be made of the existing health status of the user of hazardous substances. Are special arrangements required? E.g. for types of PPE, Is a pregnancy risk assessment required?(refer to SCP 33 this should be undertaken by the H&S Unit)**		Duration of Exposure: mins/hrs 3 hours Are there any Ethical Issues? None (Attach Consents)
Emergency Contact Names Dr Montse Ortoneda		**Tel No:** 01512312244
Description of Waste Disposal Methods: Wash it with copious water.		
Emergency Action Procedures (spillages/ leaks, first aid, fire & explosion) Any spillages should be diluted and washed away with large amount of water taking care to avoid splashing.		

Does the experiment run overnight or at weekends unattended? Has the appropriate form been completed? No
Training/Direct Supervision Requirements Only trained students should handle the chemicals.
Business Interruption (e.g. power shutdown, fire, flood) What are the Contingency Procedures for work and waste? Electricity to the fume extraction cabinet should never be cut off.
Risk Evaluation, Handling and Storage Comments Ensure good ventilation/exhaustion at the workplace. Avoid contact with skin, eyes and clothing. Avoid breathing dust. Use personal protection equipment. Wash hands.

COSHH MATERIAL SAFETY DATA

TECHNIQUE/METHOD TITLE:

Hazardous Substance (Including Organisms)	**Hazard Type**	**State e.g. solid, liquid, gas**	**Quantity used**	**Route of entry & Target Organs**	**WEL**	**Controls and Precautions**	**Disposal route, Spillage procedure Emergency procedures**
p-Dimethylaminobenzaldehyde (DMAB)	Harmful if swallowed	Solid	100g (Max)	Eyes, mouth (swallowing), respiratory system and skin		Do not breathe dust. Use personal protective equipment as required. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. Do not eat, drink or smoke when using this product. If swallowed, Call a poison centre/doctor if you feel unwell. Rinse mouth. Do NOT induce vomiting. Protect from sunlight.	Do not allow product to reach sewage system. This product may be mixed with a combustible solvent and burned in a chemical incinerator equipped with an After-burner and scrubber. This product can also be sent to an EPA approved waste disposal facility. Dilute with plenty of water. Do not allow to enter sewers/ surface or ground water. Use cleansing agent if required along with water.
Hazard Statement	・Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008. ・This substance is not classified as dangerous according to Directive 67/548/EEC.						

Description of first aid measures	· **General information:** Symptoms of poisoning may even occur after several hours; therefore medical observation for at least 48 hours after the accident. · **After inhalation:** Remove affected person to fresh air. Seek medical attention if symptoms persist. · **After skin contact**: Immediately flush with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Seek medical assistance. · **After eye contact:** Hold eyelids apart and flush eyes with plenty of water for at least 15 minutes. Get medical attention. · **After swallowing:** Immediately call a doctor. Do not induce vomiting. If conscious, rinse mouth with water ensuring that the rinse is not swallowed						
Accidental Release Measures	· ***Personal precautions, protective equipment and emergency procedures*** Wear protective equipment. Keep unprotected persons away. Avoid formation of dust. Avoid breathing dust. · ***Environmental precautions:*** Dilute with plenty of water. Do not allow to enter sewers/ surface or ground water. · ***Methods and material for containment and cleaning up:*** Use the appropriate tools to collect the material and dispose of it in an approved waste disposal container.						
Fire-fighting Measures	Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.						
Urea	Not Hazardous	Solid	100g (Max)	Eyes, mouth (swallowing), respiratory system and skin		Avoid dispersal of spilt material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas.	Put on appropriate personal protective equipment. Avoid the creation of dust when handling and avoid all possible sources of ignition (spark or flame). Prevent dust accumulation. Use only with adequate ventilation. Electrical Equipment and lighting should be protected to appropriate standards to prevent dust being exposed to hot surfaces, sparks or other ignition sources. Take precautionary measures against electrostatic discharges. To avoid fire or explosion, dissipate static electricity during transfer by earthing and bonding containers and equipment before transferring material.
Hazard Statement	No known significant effects or critical hazards						
Description of first aid measures	· **Contact with Skin** Wash the affected area thoroughly with clean water and soap. · **Contact with Eyes** Rinse with plenty of clean water for a minimum of 15 minutes; if irritation of eyes persists, obtain medical attention. · **Ingestion** Do not induce vomiting. Give patient water to drink. Obtain medical attention if more than 30ml swallowed. · **Inhalation** Remove patient from contaminated area to an area with a source of fresh air.						
Fire-fighting Measures	· **Use dry chemical powder.** Do not use water jet						

HCl	Dangerous	Liquid	100ml (Max)	Eyes, mouth (swallowing), respiratory system and skin	**1 ppm (8-hr)** **5 ppm (15-min)**	Avoid breathing dust/ fume/ mist/ vapours/ spray. Wash skin thoroughly after handling. Use only outdoors or in a well-ventilated area. Wear protective gloves/ protective clothing/ eye protection/ face protection. Wash contaminated clothing before reuse.	Keep only in original container. Absorb spillage to prevent material damage. Store in a well-ventilated place. Keep container with a resistant inner liner. Store locked up. Store in corrosive resistant stainless steel container with a resistant inner liner. Dispose of contents/ container to an approved waste disposal plant.
Hazard Statement	· May be corrosive to metals. · Causes severe skin burns and eye damage.						
Description of first aid measures	· **If swallowed:** Rinse mouth. Do NOT induce vomiting. · **If on skin (or hair):** Remove/Take off immediately all contaminated clothing. Rinse skin with water. Shower · **If inhaled:** Remove victim to fresh air and keep at rest in a position comfortable for breathing. Immediately call a POISON CENTER or doctor / physician. · **If in eyes:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor / physician.						
Fire-fighting Measures	· Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.						
TCA	Dangerous	Solid	250g (Max)	Eyes, mouth (swallowing), respiratory system and skin		Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection / face protection. Use personal protective equipment. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust	Collect spillage. Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal. Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.
Hazard Statement	· Causes severe skin burns and eye damage. · Very toxic to aquatic life with long lasting effects.						
Description of first aid measures	· **General advice** Consult a physician. Show this safety data sheet to the doctor in attendance. · **If on skin (or hair):** Take off immediately all contaminated clothing. Rinse skin with water/shower. Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician. · **If inhaled:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER or doctor/ physician. · **If in eyes:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. · **If Swallowed:** Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.						
Fire-fighting Measures	Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.						

Phosphate pH 7 Buffer	Slightly Hazardous	Liquid	1L (Max)	Eyes, mouth (swallowing), respiratory system and skin		Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapours below their respective threshold limit value. Personal Protection: Safety glasses. Lab coat. (In Case of a Large Spill: Splash goggles. Full suit. Boots. Gloves.) Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.	Keep locked up. Do not ingest. Do not breathe gas/fumes/ vapour/spray. Wear suitable protective clothing. If ingested, seek medical advice immediately and show the container or the label. Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 25°C (77°F).
Accidental Release Measures	· **Small Spill:** Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. If necessary: Neutralize the residue with a dilute solution of acetic acid. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements. · **Large Spill:** Poisonous liquid. Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapours. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of acetic acid. Finish cleaning by spreading water on the contaminated surface and allow evacuating through the sanitary system.						
Description of first aid measures	· **Eye Contact:** Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention if irritation occurs. · **Skin Contact**: Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops. Cold water may be used. · **Inhalation:** If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention. · **Ingestion:** If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.						

COMMENTS: Attach any additional hazard data, consider any substance with an allocated WEL. Use EH40 Guidance HSE

OVERALL RISK RATING

HIGH	MEDIUM	LOW
	Medium	

APPENDIX D

PUBLICATIONS

K H Joshi, A Mason, O Korostynska, A Al-Shamma'a. Milk Quality Monitoring Using Electromagnetic Wave Sensors. Sensors for Everyday Life: Environmental and Food Engineering. S. C. Mukhopadhyay, O. A. Postolache, K. P. Jayasundera and A. K. Swain. Cham, Springer International Publishing, 2017, pp. 205-227. doi:10.1007/978-3-319-47322-2_10.

K H Joshi, M Al-Mansara, A Mason, O Korostynska, A Powell, M Ortoneda-Pedrola, and A Al-Shamma'a, "Detection of Heparin Level in Blood Using Electromagnetic Wave Spectroscopy," 9th International Conference on Developments in eSystems Engineering (DeSE), Liverpool, 2016, pp. 329-334. doi:10.1109/DeSE.2016.51.

K H Joshi, A Mason, A Shaw, O Korostynska, J D Cullen and A Al-Shamma'a, "Online monitoring of milk quality using electromagnetic wave sensors," 9th International Conference on Sensing Technology (ICST), Auckland, 2015, pp. 700-705. doi: 10.1109/ICSensT.2015.7438487.

K H Joshi, P Kot, A Shaw, and S Wylie, " Determining the Dielectric Property of Milk Products for Online Quality Monitoring using Microwave Spectroscopy," 4th Faculty of Engineering and Technology Research Week, Liverpool John Moores University, Liverpool, 2018.

K H Joshi, A Shaw, A Mason, O Korostynska, and A Al-Shamma'a, "Detection of adulteration in milk products using electromagnetic wave sensors," 3rd Faculty of Engineering and Technology Research Week, Liverpool John Moores University, 2017, Liverpool, 2017, pp. 99-103. Available: https://www.ljmu.ac.uk/~/media/files/ljmu/research/centres-and-institutes/engineering-and-tech/fet_frw_proceedings_2017.pdf.

K H Joshi, A Mason, O Korostynska, A Shaw, J D Cullen and A Al-Shamma’a, “Determining overall quality of milk products using microwave spectroscopy,” 2nd Faculty of Engineering and Technology Research Week, Liverpool John Moores University, Liverpool, 2016, pp. 308-314. Available: https://www.ljmu.ac.uk/~/media/files/ljmu/research/centres-and-institutes/engineering-and-tech/fet_frw_proceedings_2016.pdf.

K H Joshi, A Mason, A Shaw, O Korostynska, J D Cullen, and A Al-Shamma'a, "Design and development of electromagnetic wave testing of milk quality: A novel approach to detect spoilage and milk types," 1st Faculty of Technology and Environment Research Week, Liverpool John Moores University, Liverpool, 2015.