Deanship of Graduate Studies

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New Application for Photopyroelectric Film in Performing Quality Analysis for Food Matrix

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Dedication

I would like to dedicate this thesis from the deep of my heart to *:*

My homeland Palestine, the land of creativity and inspiration,

Al-Quds University, the brilliant success and innovation home,

My director and colleagues in both Asma' and Rajab secondary schools , who gave me the support and the facilities to continue my research during my job.

My great parents, who always love me and never stop encouraging me and providing all kinds of support to achieve my dream.

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To all my relatives, students, friends, and teachers, all people in my life who love me and touch my heart.

I dedicate this research.

Abrar

Declaration

I certify that the thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signed: ……..

Abrar Sa'id Khaled Al-Za'tari

Date: 25/7/2018

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Abrar Sa'id Al-Za'tari

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Abstract

The Photopyroelectric film Known as polyvinylidenedifloried film (PVDF) was used to invent new technique for detecting food elements qualitatively and quantitatively. Several Food items; namely; carbohydrates, proteins, fat, minerals, vitamins, and minerals, as well several microorganisms, namely; *Salmonella, , Escherichia coli ,Staphylococcus, Pseudomonas,* yeast and mold were involved.

Several concentrations of the investigated materials were prepared in form of 0.1, 0.5, 1, 3, 5, 7, and 10%.

The PVDF found to be very effective in determining the concentrations and detecting the presence of several food elements, as in case of NaCl, $CaCl_2$, Sucrose, and Tryptophan. The detection was in term of full beam, while each item were measured at all investigated concentrations at specific wavelength. The results obtained showed very remarkable correlation between the measured food elements; NaCl, $CaCl_2$, Sucrose, and Tryptophan and the wavelength; 720, 850, 1000, and 760nm, respectively.

At the same time the measurements for microorganism, i.e. candida, found to be measurable at 950nm.

The real food samples; Eggs in form of whole egg, egg yolk, and egg white and Milk in form of raw, pasteurize, and sterilize were stored at different storage temperature. The samples tested showed huge correlation between the storage time and the measurements of the absorbance as an indicator of higher growth of microorganism by increasing storage time.

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LIST OF ABBREVIATIONS

PVDF	Polyvinylyene difluoride film
IR	Infra red radiation
Ml	MiliLeter
Nm	Nano meter
ppm	Part per billion
UHT	Ultra high tempreature
E.coli	*Escherichia coli*
µm	Micro meter
Hz	Hertz
UV	Ultra violet
HPLC	High-performance liquidchromatography
FTIR	Fourier-transform infrared spectroscopy
RMSEP	Root Mean Square Error of Prediction
ELISA	Enzyme-linked immunosorbent assay
RAST	Radioallergosorbent assay
PCR	Polymerase chain reaction
EVOO	Extra virgin olive oil
NIR	Near infrared
GC	Gas chromatography
LC	Liguid chromotography
PCA	Principal component analysis
UPLC–MS/MS	Ultra performance liquid chromatography mass spectroscopy
a.u	Arbitrary unit
DNA	Deoxyribonucleic acid
mRNA	Messenger Ribonucleic acid
RT	Room temperature
µl	Micoliter

Chapter One Introduction

1.1. **Introduction**:

BY returning to history of our universe, human spent his life looking for food, until these days human is still seeking for a good food but in better ways.

Food, is a word that can simply mean all substances people eat to sustain their life and to meet their body's basic needs, for growth, development and functions. Every single cell in our body depends on a continuous supply of calories and nutrients. So what is the components of that magical basic of people's life?!

"Food" as scientists showed is a substance consumed to provide nutritional support to organism, it contains essential nutrient such as: carbohydrate, protein, fat, vitamins and minerals. As food is made up of these nutrients which provide the essential nutrient for health, so the body cannot function properly if one or more nutrients are missing.

While Food is necessary to sustain life, this food has to be processed to insure it is microbiologically safe to eat, and also to transform unpalatable or unacceptable raw materials into attractive and desirable products, (Schascher, 2011).

Food processing as archaeological evidence that first begun in hunter-gathers societies which had cooked by using open fire for meat, fish, and vegetables. Gradually development of civilization as Egyptians societies who had developed food processing techniques, such as sun drying for meat, and grinding for cereal, so during thousands of years food processing have developed until this time with technology development, as all industry developed with electricity and technology revolutions food industry also speed in all areas, from coffee machine to food factory machinery. (Fellows, 2009)

Coupled with the speed in technology development, consumers also became more aware about their health, so consumer' demands keep changing over time. These changes range from basic considerations such as improving food safety, shelf life, and reducing wastage, to demand for

increasingly sophisticated foods having special characteristics in terms of nutritional value, palatability, and convenience. The actual product development process is determined by the interaction between consumer expectations and demand, the technical capacity of the food producer, and emerging knowledge from food science research. (Winger, 2006)

From this awareness about health and its relationship with good quality of food, which has been recognized by international governments, in the time whereworld wild nowadays in the midst of our world which is full of junk food, bad nutrient diet and overcome of deficiency disease,A healthy and balanced diet provides foods in the right amounts and combinations that are safe and free from disease and harmful substances become the most important demand.

For that reason, adequate nutrition becomes one of the most issues in public health. So before developing and implementing effective intervention programs to improve nutrition at the population level, it is important to know the nutritional situation of the target group. To assess the energy and nutrient intake of population, the nutrient intake from food consumption requires reliable data on food composition. These data are also the fundamentals of food-based dietary guidelines for healthy nutrition, containing the necessary information on food sources for different nutrients, and that information for assessment cannot be established without food composition tables and analysis.

Furthermore, food composition tables can provide information on chemical forms of nutrients and the presence and amounts of interacting components, and thus provide information on their bioavailability. For some nutrients such as vitamin A, vitamin E and niacin, the concept of equivalence has been introduced to account for differences in the availability and biological activity of different chemical forms. Although most food composition tables focus on energy, macro- and micronutrients, interest in non-nutritive components is increasing. Considering the beneficial effects of biologically active secondary plant cell compounds such as polyphenols and carotenoids, more data on these are needed. (Elmadfa, and Meyer, 2010).

On the other hand, there are a number of naturally occurring or ‘man-made’ non-nutritive substances with negative effects, and to control exposure, the main dietary sources must be

known. Another aspect is contaminants, which could have detrimental effects on consumers' health. Among these are agrochemicals, industrial pollutants reaching the food chain and substances formed during food preparation. A valid risk assessment requires data on exposure, and thus on the contents of contaminants in foods. However, these data are highly variable and may significantly differ even within narrowly confined regions. (Elmadfa, and Meyer, 2010).

From this point, to improve consumer health, specially the children and to prevent the nutrient deficiency diseases, it is necessary for government to assess the diet, food quality and to put the diet guidelines, which cannot be established without knowing the food quality and quantity.

For the purpose of that issue, food quality should be determined, food quality as Singham, Birwal and Yadav (2015) defined, the degree of excellence of food includes factors such as taste, appearance, and nutritional quality, as well as in bacteriological or keeping quality. Food quality goes hand in hand with food acceptability, and it is important that quality is monitored, both from a food safety standpoint and to ensure that the public likes a particular product and will come to select it.With this intention, food quality cannot be estimated until the food composition is determined, and that needs good methods to detect food composition, which are called food analysis techniques.

In the event that, analysis of food aimed to get information about the composition of food sample or products, investigations in food science and technology, whether by food industry, governmental agencies, or universities, often require determination of food composition and characteristics. Trends and demands of consumers, the food industry, and national and international regulations challenge food scientists as they work to monitor food composition and to ensure the quality and safety of the food supply. All food products require analysis as part of a quality management program throughout the development process, through production, and after a product is in the market. The chemical composition and physical properties of foods are used to determine the nutritive value, functional characteristics, and acceptability of the food product. The nature of the sample and the specific reason for the analysis commonly dictate the choice of analytical methods. Speed, precision, accuracy, and durability often are key factors in this choice.(Nielson, 1998)

On the other hand, another benefit of determined food quality is to reveal on food adulteration (to replace a good quality ingredient with lower quality one) in food manufactures, which can be achieved by food analysis.

Analysis of foods is continuously requesting the development of more robust, efficient, sensitive, and cost-effective analytical methodologies to guarantee the safety, quality, and traceability of foods in compliance with legislation and consumers' demands (Otles, 2014)

By the time, alternately the development and improvement of analysis techniques were started from the old or traditional methods access to the most modern instrumental analytical technique, scientists have used many techniques to analyze food, these methods can be broadly grouped into two kinds: the wet chemical analysis(traditional methods) and the non-destructive methods (Physical methods).

In the first years of the analytical chemistry the majority of the analysis were done by dividing the components of the sample that should be examined. During this process precipitation, extraction or distillations were applied. Afterwards the divided components, meant to be used for qualitative analysis, were handled by other reagents with the help of chemical reaction was used either coloured compound or changes of its boiling/freezing point or its solubility. Moreover, reactions which were applied led to variously perceptible gases (e.g.: odours) or changes in the compound's optical characteristics or optical activity. When classical analytical method is chosen for the quantitative analysis of the components (to determine its relative or absolute concentration) gravimetric or volumetric method can be used.(Kovacs., 2015).

In gravimetric measurements, the determination of the components' concentration in the given sample is led back to the changes in the mass of the examined analyte or to the mass of the precipitate that was formed with another component. In case of volumetric measurements, also known as titrimetric methods, the component which is analysed, in form of a solution, must be reacted with the reagent, already being in the standard solution and after the reaction of all the amounts of reagent in the sample, from the loss of the amount of the standard solution (from the proportional value of the stoichiometric quantity), the concentration can be determined. All of these classical analytical methods, can be used either for separating or

defining these components, are still used in several laboratories nowadays; but the number of those, who generally use these methods is slowly decreasing due to the appearance of more developed and more conveniently applicable methods of instrumental analysis. These new methods are slowly, but surely superseding the aforementioned ones, (Kovacs, 2015).

In view of old methods used the so-called "wet chemistry" have evolved into the current powerful instrumental techniques used in food laboratories. Often, modern instrumentation cannot determine results which many specific wet chemical tests provide. Wet chemistry includes basic experimentation techniques such as measuring, mixing, and weighing chemicals, conductivity, density, pH, specific gravity, temperature, viscosity, and other aspects of liquids. Wet chemistry is usually qualitative. Qualitative means to determine the presence of a specific chemical rather than the exact amount. Some quantitative techniques are used in wet chemistry, and they occur as gravimetric (weighing) and volumetric analysis (measuring), (Oltes, 2014).

For example, a traditional chemical analyses of sugars, lignin of lignocellulosics have been performed by acid hydrolysis followed by gravimetric determination of lignin and chromatographic determination of sugars, These methods can provide highly precise data, but because they are laborious, time-consuming, and, consequently, expensive to perform, sample throughput is limited. Thus, there is a great deal of interest in developing analytical tools that can be used to rapidly, inexpensively measure the chemical composition of biomass (Kelley, 2004).

At the beginning of the twentieth century scientists began to take more and more advantage of the different opportunities provided by the measured components' physical correlations. With modern methods of food analysis they developed better and better instrumental analytical methods in which they found solution for several problems of the classical analytical methods. Such physical characteristics are for example: conductivity, electrode potential, light absorption, light emission, fluorescence and the mass-charge ratio, which were started to be used for quantitative analysis. Furthermore, highly effective chromatographic and electrophoretic techniques were also used to substitute distillation, extraction or precipitation, applied to divide the mixture of components of food or food raw material samples with unusually complex matrix before the qualitative or quantitative determination. The

aforementioned new methods, used for the separation and determination of different components, are called instrumental analytical methods. It also must be mentioned that lately the rapid development of the computer and electronics industry highly contributed to the improvement and spread of the modern instrumental analytical methods. (Kovacs, 2015).

Traditionally, analytical techniques have been classified according to their working principle. For example, they can be spectroscopic (e.g., mass spectrometry (MS); nuclear magnetic resonance (NMR); infrared (IR); atomic spectroscopy (AS), biological (polymerase chain reaction (PCR); immunological techniques; biosensors), electrochemical (including also biosensors here), for separation (e.g., high-performance liquid chromatography (HPLC); gas chromatography (GC); capillary electrophoresis (CE); supercritical fluid chromatography (SFC)), for sample preparation (e.g., solid phase extraction (SPE); supercritical fluid extraction (SFE); headspace (HS); flow injection analysis (FIA); purge and trap (PAT); microwave-assisted extraction (MAE); automatic thermal desorption (ATD)), hyphenated (e.g., putting together separation and spectroscopic techniques), and so forth. Every technique provides specific information on the sample or components under study based on a specific physical-chemical interaction, and all have their own advantages and drawbacks when applied to food analysis as will be discussed below. An additional idea on the complexity ofthe number of techniques currently involved in food analysis can be obtained developing a little more one of the above sub-disciplines. Considering the case of immunological techniques,they include the following ones: enzyme immunoassay(EIA), enzyme-linked immunosorbent assay (ELISA),immunodotting, radioimmunoassay (RIA), solid-phase RIA,liquid-phase RIA, immunoradiometric assay (IRMA), fluorescence:fluorescence immunoassay, enzyme-linked fluorescentimmunoassay, fluorescence polarization immunoassay(FPIA), time-resolved fluorescence immunoassay (TRFI),and chemiluminescence immunoassay (CIA), up to 27techniques can be associated to immunological techniques(García-Cañas et al., 2012).

Advances in sample preparation aim to minimize laboratory solvent use and hazardous waste production, to save employee labor and time, and to reduce the cost per sample, while improving the efficiency of the analyte isolation. This includes the development and application of molecularly imprinted polymers in food sample analysis, the use of monoliths in

sample preparation and analysis of milk, the development of porous monolith microextraction techniques for determination of veterinary residues in food matrices by, the use of the so-called QuEChERS (quick, easy, cheap, effective, rugged, and safe) methodology for determining pesticide residues in food matrices, the application of immunoaffinity column clean-up techniques in food analysis, the development of solid-phasemicroextraction (SPME) techniques for quality characterization of food products, the application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples, and the use of liquid phase micro extraction in food analysis. Besides, some papers have focused on the description of sample preparation strategies used for the analysis of aflatoxins in food and feed, antibacterial residues in foodstuffs, or the determination of pesticides in foods. At present, new green sample preparation methods are being studied; among them, supercritical fluid extraction (SFE) and subcritical water extraction (SWE, also called accelerated solvent extraction) are among the more promising processes in food science, not only in food analysis but also for obtaining new functional food ingredients. These extraction techniques based on pressurized fluids provide higher selectivity, shorter extraction times, and are environmentally friendly, (García-Cañas et al., 2012)

Another key point, the detection and enumeration of pathogens in food and on surfaces that come into contact with food are an important component of any integrated program to ensure the safety of foods throughout the food supply chain, Microbiological analysis is also an essential tool for carrying out tests in accordance with the microbiological criteria established for each food type, as well as being essential for evaluating the actions of different management strategies based on the Hazard Analysis and Critical Control Points (HACCP) system. Microbiological analysis of foods is based on the detection of microorganisms by visual, biochemical, immunological, or genetic means, either before enrichment (quantitative or enumerative methods) or after enrichment (qualitative methods, also known as presence/absence tests). Traditional culture methods for detecting microorganisms in food are based on the incorporation of the food sample into a nutrient medium in which the microorganisms can multiply, thus providing visual confirmation of their growth. These conventional test methods are simple, easily adaptable, very practical, and generally inexpensive. Although not lacking in sensitivity, they can be laborious and depend on the

growth of the microorganisms in different culture media (pre-enrichment, selective enrichment, selective plating, identification), which may require several days before results are known. Products that are minimally processed have an inherently short shelf life, which prevents the use of many of these conventional methods. Therefore, extensive research has been carried out over the years to reduce assay time through the use of alternative methods for detecting food borne microorganisms and reduce the amount of manual labor by automating methods whenever possible, (Guillermo Lopez-Campos et al., 2012).

In this regard, anon- destructive technique has been developed to enhancement of food composition and pathogen detection with less time, cost and more accuracy, these conventional methods require laboratories, high-cost equipment, and professionals. Moreover, complicated procedures for sample preparation and long analysis times are needed thus preventing rapid detection and implementation in white electronics found in stores and homes. The limitations above have restricted their widespread use in food processing, transportation, marketing, and preservation in various food industries (Yoon et al., 2015).

However, non-destructive testing equipment can be widely used throughout the food industry. Raw material control in the field or at the factory reception, process control either online or off-line after sampling, rapid analysis of intermediate or final products in the laboratory, product development and storage testing and research are the most relevant non-destructive testing areas, In a brief definition, the qualitative and quantitative measurements in agricultural products and processed food that has been surveyed without any physical, chemical, thermal and mechanical damages to cycle back is called non-destructive test. Diversity and abundance of the parameters and qualitative features of agro-products were the most reason of Non-destructive development methods in recent four decades with the growth of the technology of the accurate measuring instruments. Non-destructive tests should not be detrimental effects on the product and it should be in order to ensure customer satisfaction of products. Various non-destructive methods have been described in table 1.1 by Aboonajmi, and Faridi in (2016).

Table 1.1. Various non-destructive methods in food industry.

Scientific basis	The method used	Measurable features
Optical	Image Processing	Size, shape, colour, outward defects
	Spectroscopic reflectance, transmission and absorption, laser spectroscopy	Sugar, acidity, soluble solid content, colour, internal and external defects, stiffness
X-Ray	imaging X-Ray And CT	The inner cavity structure, the degree of maturity
Mechanical	Vibrational excitation	Stiffness, viscoelasticity, the degree of maturity
	Sound and ultrasound	Stiffness, viscoelasticity, internal cavity, density, sugar and
Electromagnetic	MRI and NMR And NIR	Sugar, moisture content, the inner cavity
Chemical	E-nose, E-tongue	Acidity, sugar

In addition, (Narsaiah and Jha, 2011) had mentioned that Non-destructive methods for determining composition and quality include colour measurement, computer image processing, visual and NIR spectrometry, hyperspectral imaging, x-ray imaging, ultrasound, Nuclear magnetic resonance imaging (NMRI), e-nose and biosensors. These methods have the advantage of being non-destructive, fast, inexpensive (after development), and are considered suitable for online determination of many parameters simultaneously.

Because of, the previous applications have made the effective but sophisticated and costly detective technique an important tool in food matrix detection, this thesis aims to establish a technique that eliminates the laborious work. The suggested method is dependent on the measurement of a collective IR radiation absorption signal of food matrix in conjunction with photopyroelectric film (PVDF).However, this technique is used for the first time to detected food matrix and its related pathogen.

1.2 Scientific background:

1.2.1 Photopyroelectric technique:

In recent years there has been a surge of interest in investigations of materials - solids and liquids, using photothermal diagnostic techniques, which use lasers as precisely controlled optical heat sources. The development of lasers as convenient and powerful sources of localized energy has contributed greatly to the success of photothermal techniques over the conventional methods. The photothermal effects are generated by the deposition of energetic beams via direct heating provided by thermal deexcitations or by other non-thermal deexcitation processes like photoelectric, photochemical, luminescence and energy transfer processes, which result in indirect heating of the sample. If the excitation is modulated, the corresponding time and space dependent temperature variations developed in the sample gives rise to a variety of effects and most directly to temperature increase of the sample, which constitutes the basis of a distinct experimental technique. Known as photopyroelectric (PPE) effect. The pyroelectric effect consists in the induction of spontaneous, rapid polarization in a non-centrosymmetric, piezoelectric crystal as a result of temperature changes in the crystal. The measurements of the pyroelectric effect first appeared shortly before World War I. The use of pyroelectric detectors for the detection of infrared radiation was suggested early by Yeou and Chynoweth(1938,1956); however the practical pyroelectric detectors have been developed only over the last two decades. Historically, the search for pyroelectric materials has been focused on their infrared radiation detectionand their efficient high frequency response. It is surprising that the sensitivity and the unique intrinsic capability of thermal sensors based on the pyroelectric effect to respond very rapidly to thermal excitations has not been exploited with photothermal phenomena until recently, (Menon, 2001).

AsBalderas-López and Mandelis in 2002 showed that in their study by considering the one-dimensional heat diffusion problem of Fig1.1,

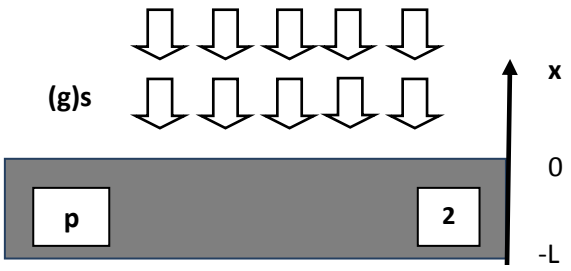


Fig.1.1. Schematic representation of the one dimensional heat diffusion model with surface absorption on the pyroelectric element.

where light, with intensity I_0 and angular modulation frequency $\omega=2\pi f$, is impinging on the upper surface of medium 2, which absorbs

light on its surface with optical absorption coefficient b,

The corresponding coupled differential equations for the one-dimensional heat diffusion are:

$$\frac{\partial^2 T_1}{\partial x^2} - \frac{1}{\alpha_1}\frac{\partial T_1}{\partial t} = 0 \qquad 0 \le x$$

$$\frac{\partial^2 T_2}{\partial x^2} - \frac{1}{\alpha_2}\frac{\partial T_2}{\partial t} = -\frac{\beta I_0 \delta(x)}{2k_2}\left[1 + e^{i\omega t}\right] - L \le x \le 0 \qquad (1)$$

$$\frac{\partial^2 T_3}{\partial x^2} - \frac{1}{\alpha_3}\frac{\partial T_3}{\partial t} = 0 \infty < x \le -L$$

where $T_i(i=1, 2, 3)$ is the temperature distribution inside medium i; $\sigma_2$is the complex thermal diffusion coefficient for medium 2, which is defined as $\sigma_2=(1+i)(\pi f/a^2)^{1/2}$, and a_2 , k_2 , and L are the corresponding thermal diffusivity, thermal conductivity and thickness, respectively. Solving this system of equations with the proper boundary conditions of heat flux and temperature continuity at all interfaces, it is easy to show that the temperature distribution inside medium 2 is given by:

$$T_2(x,t) = \frac{I_0 \beta d(1+y_{12})}{4K_2\,\sigma_2}\frac{\left[e^{\sigma_2 x} - y_{23}e^{-2\sigma_2 L}e^{-\sigma_2 x}\right]}{[1+y_{12}y_{23}e^{-2\sigma_2 L}]}e^{i\omega t} \qquad (2)$$

In this equation $\beta d$is the absorbance of the infinitesimal surface layer of material 2, where total light absorption takes place; y_{12} and $y_{23}$are thermal coupling coefficients, defined by $y_{ij}=(1-e_i/e_j)/(1+e_i/e_j)$, with e_i being the thermal effusivety of medium i. From Eq. (2) the spatially averaged temperature inside medium 2 is

$$T_2(f) = \frac{\beta I_0 d(1+y_{12})\left(1-e^{-\sigma_2 L}\right)}{4Lk_2\sigma_2^2}\left[\frac{1-y_{23}e^{-\sigma 2L}}{1+y_{12}y_{23}e^{-2\sigma_2 L}}\right]e^{i\omega t} \quad (3)$$

Considering that medium 2 is a pyroelectric transducer, with the induced voltage proportional to its thickness-averaged temperature rise , it is evident that the voltage signal can be written as0)

$$V2(f) = \frac{G(f)\beta I_0 d}{4Lk_2\sigma_2^2}\left(1 + y_{12}\right)\left(1 - e^{-\sigma_2 L}\right)\frac{\left[1-y_{23}e^{-\sigma_2 L}\right]}{\left[1+y_{12}y_{23}e^{-2\sigma_2 L}\right]}e^{i\omega t} \quad (4)$$

The function $G(f)$, called the transfer function, represents the frequency response of the sensor and the electronics. By cosidering two different materials for medium 1 (gas (g) or a transparent liquid (s) in Fig. 1), the following two equations are obtained:

$$V_p^g(f) = \frac{G(f)\beta I_0 d}{4Lk_p\sigma_p^2}\left(1 + y_{gp}\right)\left(1 - e^{-\sigma_p L}\right)\frac{\left[1-y_{pm}e^{-\sigma_p L}p\right]}{\left[1+y_{gp}y_{pm}e^{-2\sigma_p L}\right]}e^{i\omega t} \quad (5a)$$

$$V_p^s(f) = \frac{G(f)\beta I_0 d}{4Lk_p\sigma_p^2}\left(1 + y_{sp}\right)\left(1 - e^{-\sigma_p L}\right)\frac{\left[1-y_{pm}e^{-\sigma_p L}p\right]}{\left[1+y_{sp}y_{pm}e^{-2\sigma_p L}\right]}e^{i\omega t} \quad (5b)$$

Taking the ratio of these two equations we obtain

$$R(f) = \frac{V_p^g}{V_p^s} = \frac{(1+y_{gp})[1+y_{sp}y_{pm}e^{-2\sigma pL}]}{(1+y_{sp})[1+y_{gp}y_{pm}e^{-2\sigma pL}]} \quad (6)$$

It is clear that this normalization procedure eliminates the transfer function and some other parameters, which could complicate the analysis. Equation (6) involves only the thermal response of the materials under examination. When the pyroelectric element is thermally thick at large enough modulation frequencies, it is clear that R reaches the asymptotic value,

$$R_{TG} = \frac{(1+y_{gp})}{(1+y_{sp})} = \frac{1+{e_s}/{e_p}}{1+{e_s}/{e_p}} \quad (7)$$

Taking into account that gas thermal effusivity, e_g, and the effusivity of the pyroelectric sensor, e_p, usually satisfy the relation $e_g \ll e_p$, it follows from Eq. (7) that

$$R_{TG} = 1 + \frac{e_s}{e_p} \quad (8)$$

Where $e_s$is the thermal effusivity of the liquid under study. From Eq. (8) the thermal effusivity for a liquid can be found, once the asymptotic value $R_{TG}$is known, from the relation,

$$e_s = e_p\left(R_{TG} - 1\right) \quad (9)$$

The constant R_{TG} can be obtained for a given transparent liquid, by taking the signal ratio R_{TG} from the pyroelectric sensor in the thermally thick regime in two different situations: one with the bare sensor, and the other with the liquid sample in place.

1.2.2 Polyvinylidene difluoride (PVDF):

Polyvinylidene difluoride (PVDF) is an Electro-active fluoro-polymer exhibiting wide variety of characteristic mechanical and electric properties, such as piezoelectricity (the largest among the synthetic polymers), pyroelectricity, nonlinear optical property etc. It has excellent chemical resistance and solvent resistance, high abrasion resistance, high dirt shedding, low flame and smoke characteristics, low permeation to most gases and fluids (extremely important for mil-grade Micro Electro Mechanical Systems (MEMS) applications), high dielectric strength & volume resistivity, high thermal stability, resistant to gamma and e-beam radiation, high mechanical strength at elevated temperatures. It is readily process able, formable and weld able, superior melt processing characteristics. Their melt processibility along with other exceptional properties enables the polymer to stand apart from its counterparts. (Jain et al., 2015)

These properties make PVDF highly adaptable for applications in a wide range of industries, spanning aerospace, automotive, civil engineering, bio-medical and healthcare. They are extensively used in structural health monitoring systems, vibration and noise control, distance ranging and navigation, security systems. In all these systems, PVDF predominantly plays role of sensor and/or actuator. Apart from these, PVDF also plays role as the electrolyte in polymer fuel cells, insulation of the electrical harness, micromanipulators and high-energy storage devices. The five most frequently described areas of application of PVDF are- as actuators, for vibration control, in medical ultrasound, as single-element pyroelectric infrared sensors, and in strain and acceleration measurement devices. (Jain et al., 2015)

1.3 Purpose Of This Project :

The suggested method is depending on the measurement of a collective IR radiation absorption signal of food matrix in conjunction with photopyroelectric film (PVDF). IR radiation from a wideband pulsed IR source is allowed to fall on an aqueous food sample placed on top of a photopyroelectric film and the resulting signal from absorption was measured. The attained signal is then employed as a characterization of food nutrient whose absorption of IR radiation follows the food component structure. The Radiation absorbed by sample on top of PVDF initiates a Pyroelectric (PE) signal related to the Pyroelectric effect (PPE). The signal is measured as a potential difference across the PVDF and is expected to follow any variations composition of food content structure, hence allowing differentiation between different samples under study. This makes the technique practical to study food composition detection and pathogen purposes by an inexpensive easy, rapid to handle method.

2.1. Introduction:

Food analysis is important for the evaluation of the nutritional value and quality of food, and for monitoring food additives and other toxic contaminants and also the safety from food borne pathogen, for that reason in the recent decayed many new research were carried out to seek a new, rapid, more accurate and easy technique could be handled in food faculty and field

2.2. Food Analysis:

The quality and safety of food is a growing concern in recent years, for sure food has provided the most important nutrients for human to sustain life such as: carbohydrates, proteins, fat, vitamins, and minerals. However, food scientists and researchers are concerned to find new food detection methods.

2.2.1. Food sample preparation:

In analyzing food samples of the types described previously, all results depend on obtaining a representative sample and converting the sample to a suitable form.

In general, at any food matrix analysis the sample should be prepared firstly, (Cui, S. W, 2005), the sample must be prepared so as to remove substances that can interfere with analysis. For samples that are already essentially sugar solutions (juice, honey) very little sample preparation is required. Generally, samples are dried and ground first, followed by a defatting step. Drying can be done under vacuum, at atmospheric pressure, or for samples that are sensitive to heat, in a freeze dryer. Samples, once ground to a specified mesh size, are defatted using a non polar solvent such as hexane or chloroform. Low molecular weight carbohydrates can then be extracted using hot 80% ethanol. The ethanol extract will contain mineral salts, pigments, and organic acids as well as low molecular weight sugars and proteins, while the residue will mainly contain proteins and high molecular weight carbohydrates including cellulose, pectin, starch, and any food gums (hydrocolloids) that may be present. Protein is generally removed from samples using a protease such as papain. Water

soluble polysaccharides can be extracted using water and separated from insoluble material by centrifugation or filtration. Depending on the compound of interest in the sample, an enzymatic treatment with α-amylase and/or amyloglucosidase can be used to rid the sample of starch. In this case starch is hydrolyzed to glucose, which can be separated from high molecular weight polysaccharides by dialysis or by collecting the high molecular weight material as a precipitate after making the solution to 80% ethanol. Glucose is soluble in 80% ethanol while polysaccharide material is not.

2.2.2. Nutrients of food matrix:

2.2.2.1. Carbohydrates:

Carbohydrate is a major component in many foods. It is present in high concentration in cereal grains, in potatoes and in root such as cassava. Carbohydrate containing materials is the starting material for the many products like glucose syrups, modified starches, which find wide applications in food and industrial use.

Gambelli (2017) mentioned that the most used enzymatic method to measure galactose is based on its oxidation by-galactose dehydrogenase to galacturonic acid in the presence of nicotinamide-adenine dinucleotide (NAD) that is reduced to NADH, absorbance of NADH at 340 nm is calculated as the difference between the readings before and after the addition of the enzyme, galactose dehydrogenase. Although this UV method is specific and accurate, as the measurements of NADH require reading in the UV range, replacement of NAD by thio-NAD and measurement in the visible range at 405 or 415 nm can also be done. This variation allows the simultaneous quantification of D-galactose concentrations in several samples using microplate-readers, rather than UV spectrophotometers.

Regardless, other technique was used by (Kelebek et al., 2009) to determine the sugar capacity in Kozan orange juice by using HPLC system equipped with a pump system, a refractive index detector (RID-10A) for sugar analysis, which they were found that the total amounts of sugar were 120.19 g L-, and the main portions of carbohydrates in citrus fruits are the three simple sugars: sucrose, glucose, and fructose. Together, they represent about 80% of the total soluble solids of orange juice, and the ratios of sucrose:glucose:fructose are

generally about 2:1:1.which obtained that the sugar content in Kozan orange juice was agreeable to the previous data; 23.7% glucose, 25.8% fructose, and 50.5% sucrose based on total sugars present from 86 Florida Valencia orange juices.

In different way, Leopold et al. (2011) developed a new technique which dominated of Fourier-transform infrared spectroscopy (FTIR) and multivariate statistics was applied as screening tool for the quantitative determination of carbohydrates, such as glucose, fructose and sucrose, of commercial fruit juices of (orange, peach and apple) scanned by attenuated total reflectance (ATR) FTIR spectroscopy and analyzed in the 900 and 1400 cm−1 spectral range. A Principal component analysis (PCA) of the standard carbohydrate solutions were used enabled for a better understanding of the main sources of variability affecting the FTIR spectra. Partial least squares (PLS) regression were developed and used for prediction purposes, the result showed that the finger print of carbohydrate were determined of glucose, fructose and sucrose show intense characteristic bands in the region (900–1400 cm−1) of the mid-infrared spectral range. And mentioned that when the three carbohydrates are present in the same solution, strong band overlap occurs and seriously hinders individual carbohydrate quantification.

In the same manner, three types of juices (grape, pineapple, and mango) were evaluated through attenuated total reflectance–Fourier transform infrared absorbance measurements (ATR–FTIR). A calibrated method was used, different concentrations of pure sucrose (from 1 to 5 %) and registered their IR maximal wave numbers and peak intensity were plotted. The spectral peak of sucrose for each sample lies between 1057 and 1061 cm-1. DNS method was used to analyse the content of sucrose by using spectrophotometry. The wave length used for analysing is 540 nm. Also high performance liquid chromatography was used to analyse the sucrose content in the fruit juices. The results of all three experiments/techniques support each other by justifying that the mango has the high content of sucrose followed by pineapple and grape by Nair et al. (2016).

In the same token, Bureau et al. (2009) established the ATR-FTIR as a fast technique to evaluate sugars, organic acids and complementary quality traits such as fruit firmness, skin

colour, ethylene production, soluble solids and titratable acidity through the comparison with standard techniques. The potential of that method coupled with chemometric techniques based on partial least squares to assessed by comparison with currently used enzymatic determination of sucrose, glucose, fructose, malic acid and citric acid., the most suitable region was found in the range between 1500 and 900 cm1 . Good prediction performances were obtained (R2 < 0.74 and RMSEP > 18%) , the Results concerning the prediction of other quality traits such as firmness, skin colour, ethylene production, soluble solids content and titratable acidity were discussed.

2.2.2.2. Protein:

Proteins are building blocks of life and are needed in all the cells in the body to help repair cells and for growth and development. Proteins have a significant role in food, e.g. gliadin and glutenins in wheat are the basic network for our bread making.

To begin with, the traditionally methods for protein measurement the Kjeldahl method and the combustion (Dumas) method were described by Krotz et al. (2008) to determined the protein in cereal and seed .The Kjeldahl method were used based into three basic steps: first step in the analysis, to break down the bonds that hold the polypeptides together and convert them to simpler chemicals by adding strong sulfuric acid and heating the mixture to about 370ºC to 400ºC for 60 to 90 minutes oxidizes the organic material and releases ammonium ions., followed by Distillation the ammonia from the digestion mixture by raising the pH with sodium hydroxide, which changes the ammonium ions into ammonia gas. The ammonia is collected through boiling and distillation of the gas into a trapping solution of hydrochloric acid and finally .titrated the ammonia so that the quantity of distilled-off ammonia can be calculated and the amount of nitrogen in the protein determined.

Different from Krotze, an enzyme-linked immunosorbent assay (ELISA) was developed by Hefle et al. (1994) for the detection of selected peanut proteins in foods. Monoclonal antibodies against a series of allergenic peanut proteins were used as the capture antibody. Food sample extracts were then added, and polyclonal rabbit antibodies directed against roasted peanut proteins were employed as secondary antibodies. The amount of allergen

bound to the solid-phase was determined by a biotin and streptavidin-peroxidase system. Radioallergosorbent assay (RAST) inhibition studies of the food extracts were done as a comparison. The coefficient of determination for the ELISA and RAST assays was 0.85. Selected food samples were tested by RAST inhibition at another laboratory for comparison. Skin tests were done with selected samples in peanutallergic adults, and the results correlated to the ELISA and RAST inhibition results.

In other way, Screening methods for the mass spectrometric detection of caseins and whey proteins in meat products have been developed. After tryptic digestion, two α-S1-casein and two β-lactoglobulin marker peptides were measured by HPLC-MS/MS. For matrix calibrations, emulsion-type sausages with different concentrations of milk and whey protein (ppm level) were produced. The limits of detection (LODs) were below 1 ppm for milk protein and about 3 ppm for whey protein. The determination coefficients for the correlation between peak area of the marker peptides and the concentrations of milk and whey proteins were $R2 \geq 0.9899$.This study aimed to developed analytical methods that suitable for the mass spectrometric detection of caseins and whey proteins in meat products and have great potential for the further development of a multi-method for the simultaneous detection of allergens and foreign proteins in meat products. From an analytical point of view, the detection of the potential allergens milk and egg are especially interesting, as they are important foreign proteins and are not detectable by means of PCR (Jira and Schwägele, 2015).

In the event that,an assay established to be a useful tool for the food industry and regulatory agencies that wish to test foods for the presence of undeclared hazelnut allergens, enzyme immunoassay (EIA) was developed for the detection of hazelnut proteins in foods by Burton w. Blaiset L., (2001). This assay used inexpensive chicken egg yolk antibodies in a sandwich EIA format for the immunospecifice capture and detection of hazelnut proteins present in a variety of different food matrices. The assay was able to detect less than 1 ppm of hazelnut protein in most of the foods tested and did not exhibit any appreciable cross-reactivity with other nuts or food matrices.

2.2.2.3. Fat:

Fat content is determined often by solvent extraction methods (e.g., Soxhlet, Goldfish, Mojonnier), but it also can be determined by nonsolvent wet extraction methods (e.g., Babcock, Gerber), and by instrumental methods that rely on the physical and chemical properties of lipids (e.g., infrared, density, X-ray absorption). The method of choice depends on a variety of factors, including the nature of the sample (e.g., dry versus moist), the purpose of the analysis (e.g., official nutrition labeling or rapid quality control), and instrumentation available (e.g., Babcocuses simple glassware and equipment; infrared requires an expensive instrument) (Nelsien and Carpenter, 2017)

As previously mentioned, Nelsien in 2017 described Soxhlet method for fat determination, as traditional method, firstly fat is extracted, semicontinuously, with an organic solvent. Solvent is heated and volatilized, then is condensed above the sample. Solvent drips onto the sample and soaks it to extract the fat. At 15–20 min intervals, the solvent is siphoned to the heating flask, to start the process again. Fat content is measured by weight loss of sample or weight of fat removed.

Different from Nelsien, for the first time and regardless the known difficulties of analysing olive oils with E-tongue byHarzalli et al. (2018) due to their non-conductive properties and high viscosity, by Electrochemical sensor device had been used to determinethe adulteration of extra-virgin olive oil with lower-grade olive oil is a common worldwide fraudulent practice, which detection is a challenging task. The potentiometric fingerprints recorded by lipid polymeric sensor membranes of an electronic tongue, together with linear discriminant analysis and simulated annealing metaheuristic algorithm, enabled the detection of extra-virgin olive oil adulterated with olive oil for which an intense sensory defect could be perceived, specifically rancid or winey-vinegary negative sensations. The homemade designed taste device allowed the identification of admixing of extra-virgin olive oil with more than 2.5% or 5% of rancid or winey-vinegary olive oil, respectively. Predictive mean sensitivities of 84 ± 4% or 92 ± 4% and specificities of 79 ± 6% or 93 ± 3% were obtained for rancid or winey-vinegary adulterations, respectively, regarding an internal-validation procedure based

on a repeated K-fold cross-validation variant (4 folds × 10 repeats, ensuring that the dataset was forty times randomly split into 4 folds, leaving 25% of the data for validation purposes). This performance was satisfactory since, according to the legal physicochemical and sensory analysis, the intentionally adulterated olive oil with percentages of 2.5–10%, could still be commercialized as virgin olive oil. It could also be concluded that at a 5% significance level, the trained panelists could not distinguish extra-virgin olive oil samples from those adulterated with 2.5% of rancid olive oil or up to 5% of winey-vinegary olive oil.

In same manner, Luís G. Dias et al. (2014) demonstrated that a potentiometric E-tongue had the capability of discriminating monovarietal EVOO, based on the signals profiles recorded for hydro-ethanolic extracts which are riche in polar compounds that are responsible for olive oils bitterness, astringency and pungency. The E-tongue usefulness has been confirmed by analysing and successfully discriminating six monovarietal EVOO (PT cultivars: COB, MAD, VER; and ES cultivars: ARB, HOJ and PIC), with different storage times (from two months to over one year) but stored under the same conditions of light and temperature (darkness at 20 C) with predictive sensitivities greater than 97.5%. Also, the approach allowed a satisfactory predictive discrimination (61–98% sensitivity) from each ES monovarietal EVOO and the three PT ones, being EVOO of vs. VER, HOJ and PIC the olive oils more easily differentiated.

Different from, on-line NIR Spectroscopy combined with chemometric techniques (PCA, LDA and PLSR) was used for predicting water content, fat content and free acidity in olive fruits by Fernández-Espinosaand and A. J in (2016) used a three cultivar varieties of Olea europaea – Hojiblanca cv., Picual cv. and Arbequina cv. – were monitored. Five olive cultivation areas of Southern Spain (Andalucia) and Southern Portugal (Alentejo) were studied in 2011 and 2012. 465 olive samples were collected during the ripening process (non-mature olives) and compared with other 203 samples of mature olives collected at the final ripening stage. NIR spectra were measured directly in the olive fruits in the wavelength region from 1000 to 2300 nm in reflectance mode. The reference analyses were performed on the olive paste by oven drying for the moisture, by miniSoxhlet extraction for the fat content and by acid titration of the oil extracted from the olive paste. Calibrations and predictive models were developed by Partial Least Square Regression (PLSR) previous Principal Component and

Linear Discriminant analyses (PCA and LDA) were employed as exploratory and clean-up tools of data sets. The final models obtained for the total samples showed acceptable statistics of prediction with R2¼0.88, RMSEV%¼4.88 and RMSEP%¼4.98 for water content, R2¼0.76, RMSECV%¼ 19.5 and RMSEP%¼20.0 for fat content and R2¼0.83, RMSECV%¼36.8 and RMSEP%¼38.8 for free acidity. Regression coefficients were better for only one maturity state (ripe period) than for olive fruit with different composition (ripening period). All models obtained were applied to predict LQPs on a new set of samples with satisfactory results, a good prediction potential of the models.

In the same manner, The acidity, moisture and fat content in intact olive fruits were determined on-line using a NIR diode array instrument, operating on a conveyor belt By Salguero-Chaparro et al., (2013) . Four sets of calibrations models were obtained by means of different combinations from samples collected during 2009–2010 and 2010–2011, using full-cross and external validation. Several preprocessing treatments such as derivatives and scatter correction were investigated by using the root mean square error of cross-validation (RMSECV) and prediction (RMSEP), as control parameters. The results obtained showed RMSECV values of 2.54–3.26 for moisture, 2.35–2.71 for fat content and 2.50–3.26 for acidity parameters, depending on the calibration model developed. Calibrations for moisture, fat content and acidity gave residual predictive deviation (RPD) values of 2.76, 2.37 and 1.60, respectively. Although, it is concluded that the on-line NIRS prediction results were acceptable for the three parameters measured in intact olive samples in movement, the models developed must be improved in order to increase their accuracy before final NIRS implementation at mills.

As well as, olive fruit and virgin olive oil quality parameters through the direct measuring of the fruit using near infrared spectrometry (NIRS) has been investigated and the effectiveness of a portable spectrometer has been assessed. Models and calibration tests were developed using both the hexane-isopropanol extraction of individual olive fruits, and the Soxhlet extraction of olive paste. The parameters analyzed were the free acidity in olive oil, oil yield from physical extraction, oil content referring to fresh weight, oil content referring to dry matter and fruit moisture. The results indicate a good predictive potential with both

methodologies and serve to encourage improvement in the obtained models through the enlargement of the calibrations. Fruit moisture prediction models showed high accuracy (Cayuela et al., 2009)

A Characterizations of fatty acids composition in % of total methylester of fatty acids (FAMEs) of fourteen vegetable oils-safflower, grape, *silybummarianum*, hemp, sunflower, wheat germ, pumpkin seed, sesame, rice bran, almond, rapeseed, peanut, olive, and coconut oil, obtained by using gas chromatography (GC) by Orsavova et al. (2015) to study fattyacids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. The result of this saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), palmitic acid (C16:0; 4.6%–20.0%), oleic acid (C18:1; 6.2%–71.1%) and linoleic acid (C18:2; 1.6%–79%), respectively, were found predominant. The significant relationship between the reported data of total fat, SFAs, MUFAs and PUFAs intakes (% ERDI) for adults and mortality caused by coronary heart diseases (CHD) and cardiovascular diseases (CVD) in twelve countries has not been confirmed by Spearman's correlations.

Moreover, Vítová et al. in (2009) studded the physical, chemical and organoleptic properties of three types of dark chocolate with different contents of vegetable fat to identify if partial or total replacement of cocoa butter influences chosen parameters. Aroma compounds were determined by the solid-phase microextraction coupled to gas chromatography, fatty acids as methyl esters using gas chromatography, flavour and colour were evaluated sensorially, and texture by both instrumental and sensory analyses. Partial replacement of cocoa butter (up to 5% allowed by the European legislation) had only negligible effect on the monitored properties, differences between the samples were statistically insignificant ($P < 0.05$). However, total replacement of cocoa butter (as in various chocolate imitations) significantly ($P < 0.05$) influenced the properties. All the monitored physical and chemical parameters were different, high concentration (341.17 ± 13.49 mg.g-1) of elaidic acid was found here. The most evident difference was in organoleptic properties, evaluated as less expressive, atypical for good chocolate, taste and aroma with strong oleic note, which is negatively perceived by consumers.

Moreover, Bohacenko and Kopicova in (2001) studied the adulteration of of five samples of olive oils from the SIAL exhibition (Paris) and ten samples of virgin olive oils obtained on the Prague markets by additions of sunflower, soybean or rapeseed oils using a modified method of preparative LC with silica gel packed column and gradient elution with three mixtures of hexane and diethyl ether was used to separate undesirable interfering compounds in the unsaponifiable fraction before the determination of sterols using GC. Model experiments based on the determination of Δ-7-stigmastenol and campesterol (addition of sunflower and soybean oils), or brassicasterol (addition of rapeseed oil) were used to verify that this method is capable of identifying adulteration of olive oils. An elevated content of these marker sterols, in comparison with their permitted contents, enables the identification of an addition of 5–10% of the above oils to the olive oil.

Likewise, the fatty acids composition of tomato seed oil was evaluated by Gas Chromatography combined with Mass Spectrometry and the most important physical-chemicals parameters. Tomato seed oil that was used for analysis has been obtained by cold pressed extraction method. It is known that individual fatty acids can be identified by GC because of their different retention times, the samples of tomato seeds oil were esterified to bring them into a vaporous phase, transforming the fatty acid from tomato seed oil into fatty acids methyl esters. The results showed that the major component of tomato seed oil was linoleic acid (48,2%), followed by palmitic acid (17.18%) and oleic acid (9.2%), all the fatty acids were expressed in methyl esters. It can be concluded that tomato seed oil is an excellent source of essential fatty acids omega-6 (linoleic acid) and omega-9 (oleic acid) Botineștean et al. (2012).

Similarly, five cultivars (Sampion, Jupiter, Sejnovo, Elit, and Geisenheim 139) of walnuts (*Juglansregia*L.) were collected during the 2008 harvest, from Cacak, Central Serbia to study the effects of oil extraction method on the content and composition of fatty acids in the oil by using gas chromatography to determine fatty acid and HPLC for tocopherol. Two techniques of oil extraction were implemented–cold pressing and organic solvent extraction. The influence of the implemented methods on the fatty acid composition, tocopherol content as well as oxidative stability was examined. Predominant fatty acids were palmitic, oleic,

linoleic, and linolenic acids. The results showed that the oleic acid content of the oils ranged from 15.9–23.7% of the total fatty acids, while the linoleic acid content ranged from 57.2–65.1% and that of linolenic acid from 9.1–13.6%. The process of oil extraction had no significant effect on the content and composition of fatty acids in the oil the total content of tocopherols ranged from 28.40 mg/100 g to 42.40 mg/100 g of the extracted oil. The most common tocopherol in all samples was γ-tocopherol. The oil extracted using the Soxhlet method contained higher amounts of total tocopherols while the stability of the oil samples, expressed as induction period, ranged from 5.0 h to 7.1 hours. Reduced stability of the oil samples as measured by the Rancimat method was negatively correlated with the level of linolenic acid and total content of tocopherols (SoBaJiC et al., 2011).

Some of the tests involved relatively simple colour reactions such as the Baudouin reaction for sesame oil, and the Halphen test for cottonseed oil. In both cases a compound characteristic to an oil is used to determine the presence of the oil. Here again the test detected a component that today would be detected and quantified by gas chromatography (GC) or high performance liquid chromatography (HPLC). It was even possible to determine the presence of cholesterol or phytosterols, although, after separation, the identification as to which type was present depended on microscopic examination and fractional by (Jee, 2002).

Finally, A high performance liquid chromatography method with evaporative light scattering detection was developed for the analysis of oils and fats, which enabled excellent separation of major and minor triacylglycerol (TAG) species in 33 min, including regeneration of the column. The influence of the mobile phase and temperature on separation and analysis time were evaluated with a cocoa butter standard. The influence of the drift tube temperature and flow of the nebulising gas on the evaporative light scattering detector output signal was investigated by means of a response surface experimental design. Especially the flow of the nebulising gas had a profound effect on the detector signal. An optimal separation was obtained when using a 150 9 3.0 mm C18 column with 3 lm particle diameter at 20 _C and an acetonitrile/ dichloromethane gradient at 0.72 mL/min. The maximum response was attained when the ELSD detector was set at the minimum temperature (45 _C) and a gas flow of 1.2

L/min. Finally, the linearity of the detector was investigated. It was found that at very low concentrations, the signal tends to flatten towards zero, giving an underestimation for minor TAG species, especially for oils or fats with a mixed fatty acid composition. (Roeland Rombaut et al., 2009)

2.2.2.4. Vitamins:

In recent times, highly sensitive and rapid method has been developed by Ciulu et al. (2014) for the simultaneous determination of water-soluble vitamins (B2, B3, B5, folic acid, and C) in honey by LC with UV-vis detection. In their method all the vitamins were eluted in a single run within 17min. This seem to be a major breakthrough in the quantification of simultaneous determination of vitamins in honey.

Sami et al. in (2014) used HPLC to evaluate water-soluble vitamins (B2, B3, B6, B12, and C) and fat-soluble vitamins (E, K, D, A, and β-carotene) in okra pods, Okra (a vegetable by populations in Africa and Asia and particularly in Egypt) they investigated some nutritional components of okra grown in four different geographical locations of Egypt.. Results of principal component analysis (PCA) showed three clusters of varieties. The first cluster included the Dakahlia (D) and Kafr El-Sheikh (K) varieties. The second and the third clusters separated out the Suez (S) and Mansoura (M) varieties independently. The S pod showed the highest contents of vitamins B6 (49.81 μg/100 g) and E (1.47 mg/100 g) but contained the lowest contents of vitamins B3 (1.42 μg/100 g) and B12 (undetected). The K pod showed the lowest vitamin C content (11.60 mg/100 g). The M pod showed the highest contents of vitamins B3 (22.70 μg/100 g), B12 (91.20 μg/100 g), C (27.14 mg/100 g), and K (0.21 mg/100 g). The D pod showed the lowest contents of vitamins E (0.15 mg/100 g), K (0.05 mg/100 g), and B6 (11.50 μg/100 g). These findings could help develop meal planning at the community level by incorporating okra varieties with high vitamin content.

A report documents improvement validation performed on AOAC for evaluate vitamin B12 in infant formula and adult/pediatric nutritional formula. The original validation study included a range of fortified products, from infant formulas to breakfast cereals or beverages, the method has been modified to use ultra-HPLC and the calibration range extended in a multilevel

calibration curve. Detection and quantification limits were also improved by increasing the sample weight used for analysis and the reconstitution rate adapted to the requirements. The Stakeholder Panel on Infant Formula and Adult Nutritionals Test
Material Kit, designed to represent a large range of products within the category (infant formula and adult nutritionals made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein), was used to determine performance characteristics of the method. The modifications included allow now full compliance with standard method performance requirements established for vitamin B12 (SMPR 2011.005). LOQ was ≤0.01 μg/100 g, working range between 0.01 and 5.0 μg/100 g, repeatability ≤7%, and recovery in the range 90–110%. The method was granted AOAC First Action status 2014.02. Giménez (2014).

Van Wyk and Britz ,in 2010 described an extraction and detection method for vitamin B12 in dairy products and growth media cultured with Propionibacterium freudenreichii. Samples were extracted by mixing in KCN buffer (pH 4.5), autoclaving at 121 °C for 25 min, cooling and centrifugation. The resultant supernatant was syringe-filtered prior to reversed-phase HPLC analysis using a methanol-water gradient that was effective in resolving the B12 peak. The method offered excellent linearity with a regression coefficient $R > 0.998$. The limit of quantification was 0.005 μg·mL−1 sample. For samples with vitamin B12 concentrations well within the linear range of the assay, good repeatability was demonstrated for the same sample with mean concentrations of 2.62 ± 0.02 and 2.61 ± 0.02 μg·mL−1 detected on day 1 and day 2, respectively. Recovery values ranged from 98.6% to 103.2%, indicating that the extraction method ensured complete dissolution of vitamin B12 from the matrices under study. Sensitivity was enhanced by sample concentration and purification using a series of solid phase extraction steps which resulted in improved peak resolution and removal of interfering peaks. The method was validated by comparison of the HPLC results of the same sample with those obtained using traditional microbiological methods. The method is a rapid alternative to the more time-consuming microbiological assay.

In same token, (Abano and Dadzie, 2014) were determined the water-soluble vitamins(WSV): ascorbic acid (vitamin C), thiamine (B1), riboflavin (B2), niacin (B3),

panthothenic acid (B5), pyridoxine, and pyridoxal (B6), folic acid (B9), biotin(B8) , and B12 by developed HPLC coupled with electrospray ionization mass spectroscopy (ESI-MS), in fortified food samples, nutritional supplements, as well as blood plasmas, dried tropical fruits materials. The study revealed that not a single chromatographic run developed by researchers can simultaneously elute all the WSV at a time.

Engel in (2009) Developed a modern analytical methods HPLC-(UV-VIS) and HPLC-(UV-VIS)-ESI-MS/MS systems were used for the measurements and reversed phase stationary phases were applied for the separation of the vitamins. for water soluble vitamin : (C), (B1), (B2), nicotinic acid, nicotinamide, pyridoxine hydrochloride (PN), pyridoxamine-dihydrochloride (PM), pyridaxal-hydrochloride (PL), thiamine-monophosphate (TMP), riboflavine-5'-phosphate (FMN) and the piridoxal-5'- phosphate-monohydrate (PLP), pantothenic acid (B5), folic acid (B9), the sample were examined such as vitamin tablet, vitamin enriched breakfast cereal, instant cacao powder and fruit juice were commercially available products. Hydrochloric acid and metaphosphoric acid were used for the extraction of the free vitamin content of the samples. The results were obtained from this study showed that regarding the UV-VIS detection of vitamin B2 I proved that better results can be achieved at 448 nm due to the higher selectivity than at the wavelength of its absorbance maximum (λmax=270 nm), and the adequacy of the method for the determination of the bioavailable vitamin B1 (thiamine and its phosphates), bioavailable vitamin B2 (riboflavin and its phosphates) and the bioavailable vitamin B6 (pyridoxine, pyridoxal, pyridoxamine, and their phosphates) content of cereal based products.

Also, Lebiedzinska et al. (2007) have reported an HPLC method with ED and UV detection for the simultaneous determination of vitamins B1, B6, and B12. The method offers high accuracy, better repeatability and reproducibility and a relative short time for quantification of the three water-soluble vitamins assayed in food samples. The simple mobile phase and isocratic elution used to separate the thiamine, pyridoxine, pyrioxal, pyridoxamine, and vitamins B12 yielded low detection limits, good sensitivity and resolution with a minimum analysis time of 17 min

For vitamin K, Korchazhkina et al. (2006) applied tow methods of extraction of vitamin E from human milk, by use of hexane, with and without prior saponification, was compared and the analysis of milk extracts was performed by HPLC with a C_{18} column and UV detection. With both methods a significant relationship ($P < 0.01$) was obtained between levels of α-tocopherol and γ-tocopherol in human milk, although saponification resulted in higher and more consistent recovery of the internal standard, δ-tocopherol (99.6 ± 4.0%), and significantly improved the reliability of the data. The detection limit of the method (including saponification, extrac-tion, and HPLC analysis) was 0.65 μg mL^{-1} δ-tocopherol in milk. The pro-posed saponification step was a simple modification of the method, because it was performed in the same tube as the extraction, took 30 min only, and did not involve use of an inert gas. The improved method was used to measure vitamin E in several milk samples.

By use of different methods of all the vitamins for routine analysis Klejdus et al. (2004) developed an HPLC method capable of eluting both water-soluble and fat-soluble vitamins in a single run using a combined isocratic and linear gradient elution with 0.01% trifluroacetic acid (pH 3.9) and methanol as mobile phase at the flow rate of 0.7 mL/min. In their report, it was concluded that the most suitable wavelength for simultaneous determination of both water-soluble and fat-soluble vitamins is 280nm and the method is applicable to solid sample of pharmaceutical preparation (B-complex, fortified powdered drinks (multi-vitamins) and food sample.

In the same manner, Singh and Bradbury in (1988) developed an HPLC method for the simultaneous determination of αcarotene, carotene, retinol and vitamin D_2 in South Pacific root crops. Hot saponification was followed by hexane extraction, then for vitamin D_2 a clean-up procedure on an open silica gel column was applied. After testing five mobile phases, methanol- ACN-CHC13 (40:40:20) showed the best resolution in RP-HPLC, achieving the separation in the order retinol, vitamin D1, a-carotene and αcarotene within 7-8 min. UV detection was performed with a dual-wavelength detector. Average recoveries were 87%, 91%, 93% and 94% for vitamin D2, p-carotene, retinol and a-carotene, respectively. The result ahowed that Sweet potato Ipomoea batatas, taro Colocasiaesculenta, taro Xanthosomasagittifolium, giant taro Alocasiamacrorrhiza, giant swamp taro

Cyrtospermachamissonis, yam Dioscoreaalata and yam D esculenta contained no appreciable amounts of α-carotene, retinol or vitamin D2 and the amounts of β-carotene were equivalent to 0-300 μg kg-1 retinol in the fresh samples. This small amount, compared with the WHO/FAO adult RDA of 750 μg day-1, and the virtual absence of vitamin A related eye diseases in the South Pacific region, demonstrates the adequacy of greens and fruits, and fish coastal areas, in meeting the vitamin A requirement.

Too, Micali et al. (1990) reported a procedure for the determination of α, β, γ and δtocopherols,α, β, and γ -tocotrienols, β -carotene, all-trans-retinol and retinyl palmitate in the unsaponifiable fraction of butter and margarine involving HPLC on an HSSilica column with hexane-isopropanol(99.8:0.2) as eluent and programmable FLD (λ_{ex}360 nm,(λ_{em} 330-480 nm).

.Infant food such as human milk and fortified formula provide an essential source of nutrition for baby feed. This nutrition is essential for normal growth and functioning of the human body; therefore, particular attention should be paid to ensure an adequate and balanced intake of vitamins. Several methods have been developed for the analysis of vitamins in infant foods. Huang and coworkers determined vitamin K1 isomers (*cis*- and *trans*-forms) in infant formulas using UPLC–MS/MS Vitamin K1 (phylloquinone) exists naturally only in the *trans*-form. *Trans*- and *cis*-isomers are formed during UV light exposure or synthetic production of vitamin K1. This *cis*-isomer is considered to have low bioactivity. Therefore, inactive *cis*-vitamin K1 is necessary to measure individually to evaluate the true nutritional status of fortified foods. The developed method proved to be rapid and sensitive, which allows *cis*- and *trans*-vitamin K1 extracted from milk-based, rice-based, and soy-based infant formula to be simultaneously determined by UPLC–MS/MS World Health Organization in (2009).

2.2.2.5. Minerals :

Minerals are essential nutrients that are needed in small amounts to keep you healthy. Minerals do not give you energy or calories, but can help with other functions in your body.

A study have been determined the mineral elements contents in millet, Maise , Oat ,Buckwheat , sorghum and purple rice, a microwave digestion procedure optimized was applied for digesting six coarse grains. Nineteen mineral element concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The Results displayed the limits of detection (LODs) and the limits of quantification (LOQs) range from 0.0047 to 0.1250 μg/mL and 0.0155 to 0.4125 μg/mL. The relative standard deviations (RSDs) range from 0.83% to 5.03%, which showed that this proposed method was accurate and precise to detect mineral elements in coarse grains simultaneously. Correlation coefficients (r) were calculated in the range of 0.999096-0.999989. From a human nutritional point of view, buckwheat was the rich in mineral element such as Cu, Mn, K, Mg, B, Be and P. Oat contain relatively large amounts of Fe, Na, Ca, Se, Ba and Ni (Li Yang et al., 2011)

Another spectroscopy method used by Brandao (2011) ,his study established a simple and fast analytical procedure for the sequential multi-elementdetermination of Ca and Mg in dairy products employing sampling slurry and high resolution-continuum source flame atomic absorption spectrometry (HR-CS FAAS). Considering the high concentration of these species in these matrices, the analytical measurements were carried out at the secondary lines of 239.856 and 202.852 for Ca and Mg, respectively. Experiments demonstrated that the analytical curves can be established using the external calibration technique employing aqueous standards. The method allows the determination of Ca and Mg with limits of quantification of 0.038 and 0.016mg/g respectively. The results varied from 2.7 to 2.9% (all tests with n=10) and using a yogurt sample containing Ca and Mg concentrations of 1.40 and 0.13 mg g respectively. The proposed method was applied for the determination of Ca and Mg in yogurt, cow milk and milk powder samples. The samples were also analyzed after complete acid digestion and Ca and Mg determination by HR-CS FAAS. No statistical difference was observed between the results obtained by both of the procedures performed

Also, Ahmad and Roy (2009) established an ultra-sensitive and highly selective non-extractive spectrophotometric method is presented for the rapid determination of iron(II) at trace levels using 2 , 3, 4 , 5, 7-Pentahydroxyflavone (morin) as a new spectrophotometric reagent in slightly acidic solution (0.0001-0.0002 M H2 SO4) for food sample (arum (25 g),

apple (50 g), guava (50 g), and egg (1 piece)), biological materials, soil and water.The reaction is instantaneous and absorbance remains stable for over 24 h (λmax = 415 nm). The molar absorptivity was found to be 6.85 × 104 L mol −1 cm −1 . Linear calibration graphs were obtained for 0.01-10 μg mL −1 of Fe II . The stoichiometric composition of the chelate is 1:2 (Fe:morin). Large excesses of over 50 cations, anions, and complexing agents (e.g., tartrate, oxalate, citrate, phosphate, thio-urea, SCN −) do not interfere in the determination. The results of food showed that the high value of iron for psidium guajava (guava) is probably due to the involvement of a high iron concentration in the soil.

Another technique HPLC was used by (Alfawaz, 2006) to evaluate the protein, moisture, ash and lipids analysis, mineral and organic acids composition, ascorbic acid, and tocopherols of Rumex vesicarius leaves grown in the central and northern regions of Saudi Arabia were determined. This wild edible plant is locally called hummayd. The protein value was 17.1–20.1g/100g, moisture was 87.8–93.5g/100g, ash was 14.6–19.6g/100g and lipids were 3.1–3.8g/100g. The concentration of the minerals ranged from 1790 to 3680mg/100g for calcium, 2.1–3.9mg/100g for copper, 24.1–42.5mg/100g for iron, 1320–2270mg/100g for magnesium, 2710–3230mg/100g for potassium, 846–1100mg/100g for sodium and 3.7–8.8mg/100g for zinc (atomic absorption spectrophotometer analysis). The range of organic acids was 277–307mg/100g for citric, 5530–5620mg/100g for malic and 2840–3260mg/100g for oxalic acid (HPLC analysis). The chemical composition of the leaves indicates that the hummayd is a good source of minerals, a moderate source of protein and ascorbic acid, is high in oxalic acid and low in lipids and tocopherol. The high acidity and lemony flavour suggest that hummayd may be useful in the formulation of acid foods.

Another kind of spectroscopy method was used, the technique of total reflection X-ray fluorescence (TXRF) was successfully applied for the simultaneous determination of the elements Ca, K, Mn, Fe, Cu and Zn. The procedure was compared with the wet ashing and dry ashing procedures for all the elements using multivariate analysis and the Scheffe test. The technique of flame atomic absorption spectrometry (FAAS) was employed for comparison purposes and accuracy evaluation of the proposed analysis method. A good agreement between the two techniques was found when using the dry ashing and ultrasound leaching

procedures. The levels of each element found for representative samples of two onion cultivars (Yellow Granex PRR 502 and 438 Granex) were also compared by the same method. Levels of K, Mn and Zn were significantly higher in the 438 Granex cultivar, while levels of Ca, Fe and Cu were significantly higher in the Yellow Granex PRR 502 cultiva that result was carried out by Alvarez et al. (2003)

2.2.3 Food Microbiology:

2.2.3.1 Salmonella:

A study reported an approach to enable rapid concentration and recovery of bacterial cells from aqueous chicken homogenates as a preanalytical step of detection and development of an automated cell concentration instrument based on cross-flow microfiltration. A polysulfone hollow-fiber membrane module having a nominal pore size of 0.2 μm constitutes the core of the cell concentration instrument. The aqueous chicken homogenate samples were circulated within the cross-flow system achieving 500- to 1,000-fold concentration of inoculated *Salmonella enterica* serovar Enteritidis and naturally occurring microbiota with 70% recovery of viable cells as determined by plate counting and quantitative PCR (qPCR) within 35 to 45 min. These steps enabled 10 CFU/ml microorganisms in chicken homogenates or 10^2 CFU/g chicken to be quantified. Cleaning and sterilizing the instrument and membrane module by stepwise hydraulic and chemical cleaning (sodium hydroxide and ethanol) enabled reuse of the membrane 15 times before replacement. This approach begins to address the critical need for the food industry for detecting food pathogens within 6 h or less by Xuan Li et al. (2013)

In addition, Rajtak et al. (2001) developed a two-step real-time SYBR Green I multiplex polymerase chain reaction (PCR) assay with melting curve analysis for rapid detection of 19 Salmonella serotypes frequently encountered in humans, animals, and animal-associated meat products within the European Union. The first-step single-tube reaction (Multiplex PCR I), consisting of five primer pairs, classified an initial test panel of eight Salmonella serotypes into five groups on the basis of characteristic amplicon melting temperatures produced by each strain. Following designation into groups, two subsequent triplex reactions (Multiplex PCR II-G1 and II-G3) allowed for further identification of five Salmonella serotypes by their melting

peak temperatures. Primers for serotype differentiation were designed to target the genes encoding either phase 1 and 2 flagellar antigens fliC and fljB or unique serotype-specific loci. In addition, the assay simultaneously screened for the presence of the ampicilin-amoxicillin, chloramphenicol-florfenicol, streptomycin-spectinomycin, sulfanomides, and tetracycline (ACSSuT)-type multidrug resistance pattern, indicated by the floR gene, and for the Salmonella virulence plasmid encoded by the svp operon in Salmonella serotype Typhimurium. The established multiplex assays were successfully tested on 97 isolates, comprising 37 distinct Salmonella serotypes and 12 non-Salmonella strains. The two-step assay correctly detected 19 of 37 Salmonella serotypes and all non-Salmonella strains produced negative results.

Bohaychuk et al. (2005) used a rapid and molecular technologies such as enzyme-linked immunosorbent assay (ELISA), PCR, and lateral flow immunoprecipitation to reduce the time and labor involved in screening food products for the presence of pathogens. These technologies were compared with conventional culture methodology for the detection of Salmonella, Campylobacter, Listeria, and Escherichia coli O157:H7 inoculated in raw and processed meat and poultry products. Recommended protocols were modified so that the same enrichment broths used in the culture methods were also used in the ELISA, PCR, and lateral flow immunoprecipitation assays. The percent agreement between the rapid technologies and culture methods ranged from 80 to 100% depending on the pathogen detected and the method used. ELISA, PCR, and lateral flow immunoprecipitation all performed well, with no statistical difference, compared with the culture method for the detection of E. coli O157:H7. ELISA performed better for the detection of Salmonella, with sensitivity and specificity rates of 100%. PCR performed better for the detection of Campylobacter jejuni, with 100% agreement to the culture method. PCR was highly sensitive for the detection of all the foodborne pathogens tested except Listeria monocytogenes. Although the lateral flow immunoprecipitation tests were statistically different from the culture methods for Salmonella and Listeria because of false-positive results, the tests did not produce any false negatives, indicating that this method would be suitable for screening meat and poultry products for these pathogens.

Similarly, Baylis et al. (2000) were compared the ability of four rapid methods and a standard cultural method to detect low levels of heat-injured cells of Salmonella typhimurium in ice cream and skimmed milk powder. Each sample was tested by a novel broth method (S.P.R.I.N.T. Salmonella; Oxoid), a commercial polymerase chain reaction (PCR) test, an automated ELISA combined with an immuno-concentration step and an ELISA-based dipstick test . The detection of Salmonella in samples contaminated with low levels (< 10 cfu 25 g−1) was significantly greater with the novel broth method than with the other methods ($P \leq 0{\cdot}01$). At contamination levels >10 cfu 25 g−1, there was no significant difference between the methods except for the novel broth method and a dipstick-based immunoassay ($P \leq 0{\cdot}05$). The novel broth method, S.P.R.I.N.T. Salmonella, which incorporates a specifically formulated peptone and Oxyrase® combination followed by the timed release of selective agents into the recovery medium, was shown to improve the rate of detection of low numbers of injured cells of Salmonella after 24 h enrichment.

Favrin et al. (2003) applied a novel immunomagnetic separation (IMS)-bacteriophage assay to the detection of Salmonella enteritidis in artificially inoculated skimmed milk powder, chicken rinses, and ground beef. In all food types tested, the IMS-bacteriophage assay was able to detect an average of 3 CFU of S. enteritidis in 25 g or ml of food sample. Total assay time including pre-enrichment is about 20 h. The results indicate that the IMS-bacteriophage assay is a rapid and sensitive means of detecting S. enteritidis in these foods. The assay was successfully adapted to the detection of Escherichia coli O157:H7 and was able to detect E. coli in ground beef at the lowest inoculation level tested, 2 CFU/g. The assay was also adapted to the simultaneous detection of S. enteritidis and E. coli. The results indicate that the IMS-bacteriophage assay shows promise for the simultaneous detection of these pathogens, but further development work would be necessary to improve sensitivity and produce reliable results at low inoculation levels.

The ability of the Bactigen Salmonella Shigella (BSST), the Microscreen (MS), and the Spectate (SPECT) latex agglutination kits to detect Salmonella in pure cultures and in naturally contaminated foods was examined. Of 190 Salmonella strains tested, the MS, BSST, and SPECT systems correctly identified 89.5, 81.6, and 66.3% of the test cultures,

respectively. The sensitivity of SPECT increased to 92.7% when only strains belonging to the targeted serogroups (somatic A to E plus G) and strains harboring the Vi antigen were considered. The lack of specificity of the MS (3.4%), SPECT (17.0%), and BSST (33.9%) systems with 59 cultures of nonsalmonellae varied widely, with Citrobacter freundii and Escherichia coli accounting for many of the false-positive reactions. Examination of foods according to the prescribed MS and SPECT analytical test protocols identified respectively, 18 (75%) and 19 (79.2%) of the 24 food samples found to contain Salmonella spp. by a standard cultural method. Although instructions with the BSST kit indicate that the product is intended for the analysis of clinical samples, the system nevertheless identified 21 (87.5%) contaminated food samples under homologous MS and SPECT test conditions. The concurrent use of TBG43 with enrichment media recommended by kit manufactures enhanced the sensitivities of MS (83.3%), SPECT (91.7%), and BSST (91.7%). Attempts to effect greater method brevity through the application of latex kits at various stages of the standard cultural procedure were counterproductive. D'aoust et al. (1991).

2.2.3.2. E. coli :

Kiranmayi and Krishnaiah (2010) subjected 250 of beef, mutton and chicken samples to PCR and used cultural methods to check for the presence of Escherichia coli O157:H7. Primers for hlyA, stx1 & 2 genes were used for the detection of Escherichia coli O157:H7 and shiga toxins respectively. Out of 250 samples, 27 showed presence of Escherichia coli O157:H7 (5 beef, 6 beef swabs, 2 mutton, 12 mutton swabs and 2 chicken out of 50 samples each) by PCR whereas only 11 samples (one beef, 2 beef swabs, 1 mutton, 6 mutton swabs and one chicken sample) were positive by cultural method.

Moreover, Cagney et al. (2008) investigated the prevalence and numbers of Escherichia coli O157:H7 in minced beef and beef burgers in supermarkets and butcher shops in the Republic of Ireland. Fifteen samples were collected quarterly from each of 26 counties over a 13-month period. All samples (1533) were directly plated on SMAC, and enriched in mTSB with novobiocin, extracted by immunomagnetic separation (IMS), plating onto SMAC-CT agar and finally confirmed by PCR.

In the same manner Gannon et al. (1992) described the polymerase chain reaction (PCR) in detection of Shiga-like toxin (SLT)-producing Escherichia coli (SLT-EC) in ground beef. a ground beef samples were inoculated with SLT-EC strains 319 (O157:H7; SLTI and SLTII), H30 (O26:H11; SLTI), and B2F1/3 (O91:H21; SLTII variants VT2ha and VT2hb) and cultured in modified Trypticase soy broth for 6 h at 42 degrees C, an initial sample inoculum of as few as 1 CFU of these SLT-EC strains per g could be detected in PCR assays with DNA extracted from the broth cultures.

In other way, a comparison between rapid enzyme-linked immunosorbent assays (ELISAs) in beef products and immunomagnetic separation along with selective media to detect E. coli O157 on hides was used to detection of Escherichia coli O157. Naturally infected hide and fecal samples were subjected to both the immunomagnetic separation method and ELISAs for the detection of E. coli O157. Additionally, E. coli O157 inoculated and noninoculated ground beef and beef briskets were used to simulate meat and carcass samples. When comparing the detection results from the ELISAs to the immunomagnetic separation method, poor agreement was observed for fecal samples. The results showed that immunomagnetic separation is the best available method, these data suggest that the ELISAs are not useful in detecting E. coli O157 from hide or fecal samples. However, when ELISAs are used on ground beef and beef brisket samples they can be used with a high degree of confidence, (Thompson et al., 2007)

2.2.3.3 Staphylococcus:

Rodríguez et al. (2016) developed a SYBR Green-based real-time PCR (qPCR) procedure for detection of enterotoxin-producing *Staphylococcus* spp. in meat products .For this, a specific primer pair based on conserved regions of enterotoxingenes was designed for detecting most of the described staphylococcal enterotoxins. No cross-reactivity with other microorganisms or non-enterotoxin-producing *Staphylococcus* spp. was detected. The detection limits of the assay were about 2–40 cfu/g for artificially contaminated meat products after 8 h enrichment period at 30 °C. Total time for assay completion was approximately 12 h.

EI-Jakee et al. *(2013)* investigated PCR to detect the occurrence of enterotoxigenic *S. aureus* among the examined food samples, 250 food samples (milk samples, white soft cheese

samples, yoghurt samples, meat and meat products and chicken products) were investigated .the results showed that 127 isolates were identified as *Staphylococcus* species (50.8 %). 32 *S. aureus* isolates were identified from the examined samples with an incidence of 12.8%. The highest isolation rate was observed in raw milk samples (56%) followed by yoghurt samples (22%), chicken products (6%), white soft cheese samples and pasteurized milk samples (4% each) then meat and meat products samples (2%).

In the same token, Wilson et al. in (1991) used also polymerase chain reaction to amplify the staphylococcal enterotoxin B and C genes (entB and entC1) and the staphylococcal nuclease gene (nuc)in dried skimmed milk. Two sets of primers ("nested primers") were found to be necessary for the detection of low copy numbers of purified DNA in diluent. These allowed detection of ca. 1 fg of purified target DNA, while 100 pg was required before detection of entB, entC1, and nuc with single primer pairs was possible. With nested primers, enterotoxigenic Staphylococcus aureus cells could be detected in artificially contaminated dried skimmed milk samples at levels of ca. 10(5) CFU ml-1 within 8 h. No cross-reaction was observed between the highly homologous entB and entC1 genes. The method showed total specificity for entC1 when tested against a wide variety of other bacteria.

Unlike the previous studies, a 20 samples of milk collected from restaurants have been studied for the presence of *S. aureus* enterotoxigenic strains. All the isolates from milk samples have been analysed by liquid chromatography-coupled with diode array detector for the rapid identification and quantification of SEB as intact protein. Limit of detection and limit of quantification values were 0.5 and 1 μg/mL, respectively. *S. aureus* was found in 35% of analysed samples but only one of them was an enterotoxigenic strain, which produced staphylococcal enterotoxin B at levels of 3.6 μg/mL (Sospedra et al., 2012)

Even though, Callahan et al. (2006) developed mass spectrometry to identify a protein toxin, staphylococcal enterotoxin B (SEB), in a model food matrix, apple juice. The approach employs ultrafiltration to remove low molecular weight components from the sample, after which the remaining high molecular weight fraction, containing the protein, is digested with trypsin. The tryptic fragments are separated from residual biopolymers and analyzed by liquid

chromatography−electrospray mass spectrometry. The background is still sufficiently complex that tandem mass spectrometry (MS/MS) is used to confirm the identity of target peptides. Limits of detection are 80 ng of SEB for MS and 100 ng for full scan MS/MS, using a tryptic fragment as the analytical target.. The presence of SEB can be confirmed at concentrations as low as 5 parts-per-billion by increasing the size of the sample to 10 mL. The method is applicable to the detection of SEB in other water-soluble food matrixes.

Conversely, anew immunoenzymatic assay (IEA) for the identification of Staphylococcus aureus strains of both human and animal origin was compared with rapid commercial kits. The sensitivities and specificities of the commercial kits varied from 90.2 to 96% and 90.8 to 93.7%, respectively. The IEA did not give any false-negative or false-positive results, while commercial kits gave high percentages of false-positive results among clumping factor-positive non-S. aureus strains. The IEA is particularly useful for isolates for which identification is doubtful, for large-scale epidemiological studies, and for identifying isolates from animals as S. aureus (Guardati et al., 1993).

2.2.3.4.Pseudomonas :

Vijayakumar et al. (2016) performed the Polymerase chain reaction (PCR) to verify the utility of this technique in detecting low levels of microbial contamination in quality control of ophthalmic viscosurgical devices (OVDs). Universal and specific pri-mers (oprL) were applied to identify *Pseudomonas aeruginosa* as one of the objectionable microorganisms in pharmaceutical products. Samples of hydoxypropylmethylcellulose, sodium hyaluronate inoculated with defined number of *Pseudomonas aeruginosa* cells and subsequently exposed to boiling for 15 minutes for releasing DNA materials from the contaminants. The treated samples were subjected to PCR amplification. Agarose gel electro-phoresis revealed amplified fragments as predicted, with no interference from the products and other environ-mental bacterial and fungal strains included in the study. The minimum detection limit of *Pseudomonas aerugino-sa* DNA was 2.1 ng. In contrast to conventional culturing methods that require mean of 5 – 6 days for identification of *Pseudomonas aeruginosa*, the entire mentioned PCR assay lasted about 4 – 5 hours.

2.2.3.5 Fungus :

2.2.3.5.1. Mold :

A rapid, quantitative, inexpensive, and efficient method was developed to determine aflatoxins in corn, corn meal, popcorn, rice, wheat, cottonseed, and peanuts. Samples are ground and extracted with methanol–water (80 + 20). This method can quantify aflatoxin from 5 to 5000 ppb without dilution and was linear when applied to samples of noncontaminated corn spiked at 0 to 5000 mg aflatoxin B1/g. Correlation coefficients of the method with LC for multiple analyses for corn, cottonseed, and peanuts were 0.999, 0.995, and 0.980, respectively. Individual analyses may be conducted in less than 5 min, and grouping of samples is unnecessary. The sensitivity of the method for corn is 5 ppb and the fluorometer, under the operating conditions, has a limit of detection of 0.6 ng aflatoxin B1(Malone et al., 2000)

Feldsine et al. (2003) used the SimPlate Yeast and Mold–Color Indicator method (Y&M–CI), for enumerating yeasts and molds in foods frozen corn dogs, nut meats, frozen fruits, cake mix, cereal, and fresh cheese. Nut meats, frozen fruits, and fresh cheese were naturally contaminated.Test portions were prepared and incubated according to the conditions stated in both the BAM and ISO methods. Seventeen laboratories throughout North America and Europe participated in the study. Three method comparisons were conducted. In general, there was <0.3 mean log count difference in recovery between the SimPlate method and the 2 corresponding reference methods. Moreover, mean log counts between the 2 reference methods were also very similar. The repeatability (sr) and reproducibility (sR) standard deviations were comparable between the 3 method comparisons. These results indicate that the BAM method and the SimPlate method are equivalent for enumerating yeast and mold populations in foods. Similarly, the SimPlate method is comparable to the proposed ISO method when test portions are prepared and incubated as defined in the proposed ISO method..

2.2.3.5.2. Yeast:

Wan et al. (2006) developed real-time PCR method for detecting spoilage molds during screening of raw materials and final product quality control analysis. The 18S rRNA gene was used to develop PCR primers and probe. With this set of primers and probe, less than 1,000 mold cells per milliliter of orange juice (10 cells per reaction) were detected with the real-time

PCR system within 6 to 7 h. No cross-reactivity was found with other common foodborne bacteria, yeasts, or food ingredients. This technique is significantly faster than current detection and identification procedures, which take from days to weeks.

Coupled with Wan, Bleve et al. in (2003) used reverse transcriptase PCR (RT-PCR) and real-time RT-PCR assays to detect and quantify actin mRNA from yeasts and molds in contaminated yogurts and pasteurized fruit-derived products. Universal primers were designed based on the available fungal action sequences, and by RT-PCR they amplified a specific 353-bp fragment from fungal species involved in food spoilage. From experiments on heat-treated cells, actin mRNA was a good indicator of cell viability: viable cells and cells in a nonculturable state were detected, while no signal was observed from dead cells. The optimized RT-PCR assay was able to detect 10 CFU of fungi ml^{-1} in pure culture and 10^3 and 10^2CFU ml^{-1} in artificially contaminated yogurts and pasteurized fruit-derived products, respectively. Real-time RT-PCR, performed on a range of spoiled commercial food products, validated the suitability of actin mRNA detection for the quantification of naturally contaminating fungi. The specificity and sensitivity of the procedure, combined with its speed, its reliability, and the potential automation of the technique, offer several advantages to routine analysis programs that assess the presence and viability of fungi in food commodities.

On other hand, Kümmerle et al. (1998) used Computer-based Fourier-transform infrared spectroscopy (FT-IR) to identify food-borne, predominantly fermentative yeasts. Dried yeast suspensions provided the films suitable for FT-IR measurement. Informative windows in the spectrum were selected and combined to achieve optimal results. A reference spectrum library was assembled, based on 332 defined yeast strains from international yeast collections and our own isolates. All strains were identified with conventional methods using physiological and morphological characteristics. In order to assess identification quality, another 722 unknown yeast isolates not included in the reference spectrum library were identified both by classical methods and by comparison of their FT-IR spectra with those of the reference spectrum library. Ninety-seven and one-half percent of these isolates were identified correctly by FT-IR. Easy handling, rapid identification within 24 h when starting from a single colony, and a high

differentiation capacity thus render FT-IR technology clearly superior to other routine methods for the identification of yeasts.

2.3. Food Quality:

Results of Non-destructive quality tests provided a potential for quick on line testing through applying deferent methods. This part of paper will show the some of these different application on non destructive quality testing techniques for some food samples.

2.3.1 The use of NIR:

A relatively new finding for non-destructive taste testing were proven to be successful by using the NIR. Abu-Khalafand Bennedsen (2002) used the NIR technology for testing the plum taste. They invested the technology of NIR spectrometer by implementing photo diode array (PDA) detector to classify two varieties of which had the same ratio of soluble solid contents (SSC) andacidity. The optical reflectance within NIR range (700-1100 nm) proven to provide high potential for plum tasting with around 92.8% accuracy.

On the other hand, the acidity, moisture, and fat content in intact olive fruits were determined on-line using a NIR diode array instrument, operating on a conveyor belt. Several preprocessing treatments such as derivatives and scatter correction were investigated by using the root mean square error of cross-validation (RMSECV) and prediction (RMSEP), as control parameters. The results obtained showed RMSECV values of 2.54–3.26 for moisture, 2.35–2.71 for fat content and 2.50–3.26 for acidity parameters, depending on the calibration model developed. Calibrations for moisture, fat content and acidity gave residual predictive deviation (RPD) values of 2.76, 2.37 and 1.60, respectively. Although, it is concluded that the on-line NIRS prediction results were acceptable for the three parameters measured in intact olive samples in movement (Lourdes Salguero-Chaparro, 2013)

Never the less, investigated the NIR as a rapid and non-destructive method to determine soluble solid content (SSC) in intact jaboticaba [Myrciariajaboticaba (Vell.) O. Berg] fruit were carried out by Nathália Cristina Torres Mariani, et al. in 2014. The aim of their study was to build the calibration models through evaluate the potential of near-infrared reflectance spectroscopy. Multivariate calibration techniques were compared with pre-processed data and

variable selection algorithms, such as partial least squares (PLS), interval partial least squares (iPLS), a genetic algorithm (GA), a successive projections algorithm (SPA) and nonlinear techniques (BP-ANN, back propagation of artificial neural networks; LS-SVM, least squares support vector machine).

Moreover, Maria Eugenia Mora-Ruiz (2017) assessed the polar phenolic compounds of virgin olive oil by using NIR and mid-IR spectroscopy and their impact on olive oil quality. They combined the Partial Least Square (PLS) regression with analytical determination of polar phenolic compounds measured by HPLC in different varieties were used to generate calibration and validation models in order to be able to predict the content and profile of these minor compounds, and thereby the quality of the product. Satisfactory multivariate test set validation algorithms were obtained for virgin olive oil (VOO). Contrary to NIRS, the chemometric analysis of the mid-IR spectra gave no satisfactory validation.

2.3.2 The use of image processing method (IPM):

FarshadVesali et al. (2011) investigated the moisture content as an important quality feature that directly influences storability of fruits and vegetables. One cultivar of apple fruits were studied with image processing method (IPM). The image-processing method was used to obtain an index that portends the apples' density. Furthermore, wrinkles of some part of apples' skin were measured as well. The neural network estimated moisture content of apples with higher accuracy. These procedures appeared to be a good method for the assessment of apples' moisture content, non-destructively.

2.3.3 The use of computer vision system (CVS):

Yoshio Makino et al. in 2016, used the computer vision system (CVS) to grade Mangoes on theBasis of Peel Color Measurement. Red peel was selected using two types of color space values at chroma = 22 and hue angle = 52˚. Eighteen out of 25 fully-ripened fruits were graded as "excellent," determined by the share of red area per fruit being in the range of 80% - 100%.

In contrast, all green-mature fruits were graded as "fair," where the share of red area per fruit was <30%. If the threshold for the share of the red area on the peel is set between 10% (maximal green-mature fruits) and 18% (minimal fully-ripened fruits), automatic removal of

green-mature fruits on a grading line is feasible. They found that the CVS was effective for nondestructively assuming anthocyanin dye concentration.

2.3.4 The use of Hyperspectral Imaging (HI):

Chu Zhang et al. (2017) measured the spinach pigments content during storage using hyperspectral imaging with chemometrics. Hyperspectral imaging covering spectral range of 874–1734 nm was used to measure spinach leaf pigments content (chlorophyll-a (Chla), chlorophyll-b (Chlb), total chlorophyll (tChl), carotenoids (Car)). The overall results indicated that hyperspectral imaging could be used for spinach leaf pigments content measurement, providing an alternative for real-world on-line vegetables quality monitor.

2.3.5 The use of X-ray:

Sheng-Xiang Wang, et al. (2017), used the grating-based X-ray imaging to study a fresh cherry tomato and a dried umeboshi. are imaged by using X-ray. The scattering image of the cherry tomato, and the wrinkles of epicarp in the refraction image of the umeboshi, are shown distinctly. The results confirm that grating-based X-ray imaging is of great potential in non-destructive fruit testing.

Chapter Three Materials, Methods, and Methodology

3.1 Introduction:

The analysis of food has grown rapidly in last few years; Food analysts have derived a great importance in helps to obtain information about the composition, appearance, texture, flavor, shelf life, of food for costumer, food industry, food science researcher and also government agency. Most analytical techniques used in food analysis required isolation of the food component. The original properties of the product are destroyed during sample preparation and analysis. Such analyses are expensive, time consuming, and require sophisticated instrumentation.

In General, a lot of research and development work has been done on food analysis and quality. The traditional methods are sensory evaluation, chemical analysis, and microbiological analysis. A modern approach in technical sense is optical techniques, especially the multispectral methods in visual and near-infrared wavelengths. In this research, the purpose is to go through the use of a nondestructive, fast, real-time, technique in food analysis.

This chapter provides an insight into the experimental procedures and techniques used and present essential information regarding the most required elements to conduct the right investigation, namely, materials, methods, and methodology.

3.2. Materials:

3.2.1. Processing Materials:

3.2.1.1. Photopyroelectric Cell :

This investigation based on devices were locally made in AlQuds University. Where the setup of this experiment were established in the laser laboratory at Al-Quds University. Materials and apparatus involved in this investigation included:

1. Perspex:bought from local market.
2. IR source:in form of different width wirse that been bought from local market
3. PVDF: bought from Goodfellowcompany (London, UK).

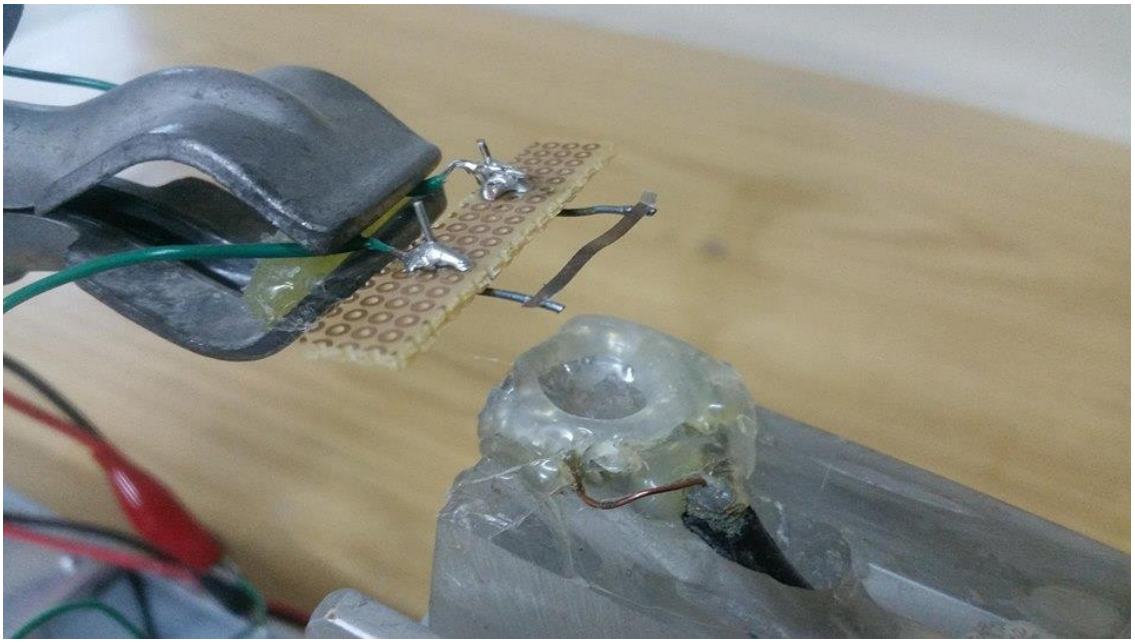


Figure 3. 1:Photo of PVDF film placed on the top of Perspex directly under IR source.

4. IR filters in different wave length (1000 nm, 950 nm, 850 nm, 760nm and 720nm) obtained from (Amazon Fulfilment Services, USA)

Figure 3.2: Photo of IR filters in different wave length (1000 nm, 950 nm, 850 nm, 760nm and 720nm).

5. Power frequency generator: with adjustable frequency (PHYWE Com, UK)

Figure 3.3: Photo of Power frequency generator.

6. Lock-in Amplifier:(PHYWE Com, UK)

Figure 3.4: The Lock-in Amplifier.

3.2.2. Analysis Materials:

3.2.2.1. Food Nutrients material:

Materials used in this investigation were contained pure food nutrient, food microbes, and real food matrix (milk, oil, butter, and egg)

However, the following materials involved in the investigation are:

- **Carbohydrate:**

1. Sucrose was used as a solution prepared in different concentration (0.1%, 0.5%, 1%, 1.5%, 3%, 5%, 10%) obtained from local supermarket.

❖ **Protein:**

1. Pure albumin crystals used as a solution prepared in different concentration (0.1%, 0.5%, 1%, 3%, 5%,7%, 10%) obtained from biology lab in al Quds University.
2. Threonine powder (98%) prepared in 0.5 g/ 10 ml distilled water, obtained from Chechen farmer
3. Lysine prepared in 0.5 g/ 10 ml distilled water, obtained from Chechen farmer
4. Methionine prepared solution of 0.5 g/ 10 ml distilled water, obtained from chechen farmer
5. Tryptophan prepared solution of 0.5 g/ 10 ml distilled water, obtained from chechen farmer

❖ **Vitamin:**

1. Vitamin $B_{6,}$ prepared solution of 5% obtained from Bet Jala Company for drug and supplement.
2. Vitamin $B_{1,}$ prepared solution of 5% obtained from Bet Jala Company for drug and supplement.
3. Vitamin $B_{9,}$ prepared solution of 5% obtained from Bet Jala Company for drug and supplement.

❖ **Fat**:

1. Sunflower oil, prepared solution of 2% and 10% in chloroform, obtained from local supermarket.
2. Cow butter , prepared solution of 2% and 10% in chloroform, obtained from local supermarket

❖ **minerals :**

1. Table salt (NaCl) was used as s solution prepared in different concentration (0.1%, 0.5%, 1%, 1.5%, 3%, 5%, 10%)
2. $Cacl_2$ was used as a solution prepared in different concentration (0.1%, 0.5%, 1%, 1.5%, 3%, 5%, 10%)
3. Ferrous powder, prepared solution of 5% obtained from Bet Jala Company for drug and supplement.

4. Sodium chloride powder, prepared solution of 5% obtained from Bet Jala Company for drug and supplement.

Figure 3.5. Photo of different food sample solution prepared in the laboratory.

❖ **Real food sample:**

Milk: Fresh milk, Pasteurized milk, UHT milk, was purchased from local supermarket.

Egg, was purchased from local supermarket.

3.2.2.2. Food Microbes:

Salmonella, , Escherichia coli ,Staphylococcus, Pseudomonas, yeast and mold, isolated cultures has been obtained from microbiology lab in department of medical laboratory science ,faculty of health professionals at Al-Quds university, and different dilutions were prepared as addressed in this investigation.

3.2.3. Chemical structure of materials :

3.2.3.1. Food nutrients :

Table 3.1.: Chemical formula of carbohydrate and protein in food .

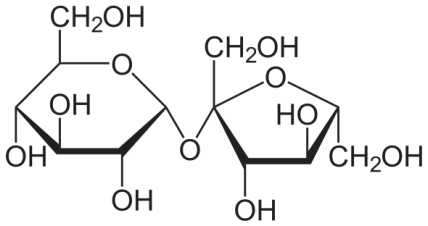
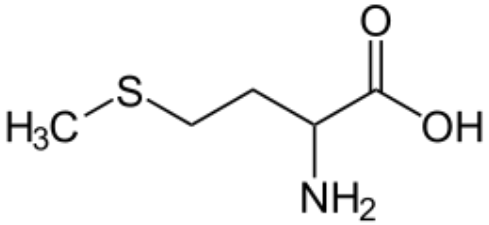
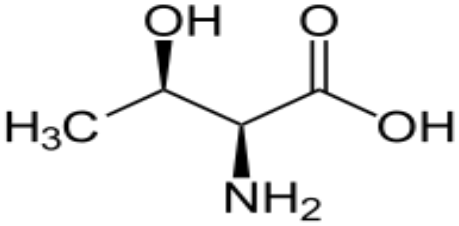
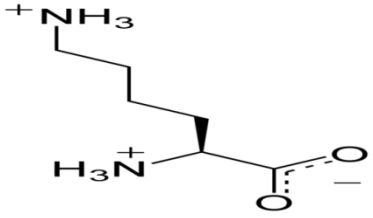
Name of materials	Chemical formula
1. Carbohydrate: sucrose	
2. Proteins : **a. albumins**	
b. Amino acids **Methionine**	
Threonine	
Lysine	

Table 3.2 : Chemical formulas of fat and vitamins in food .

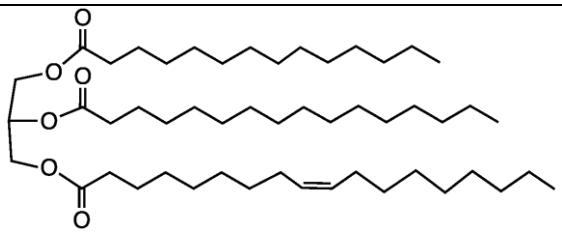
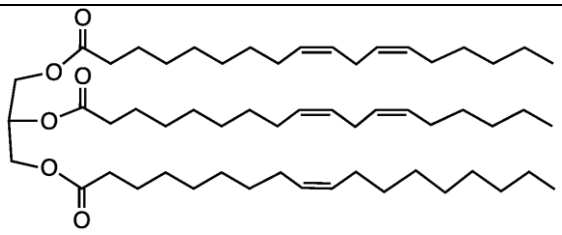
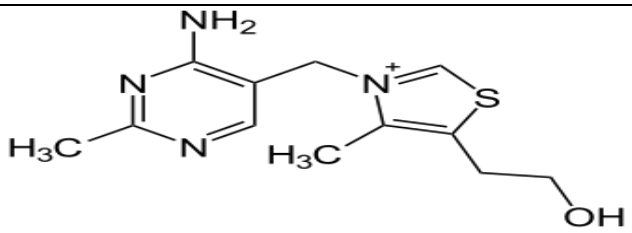
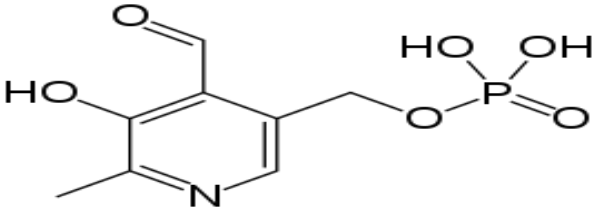
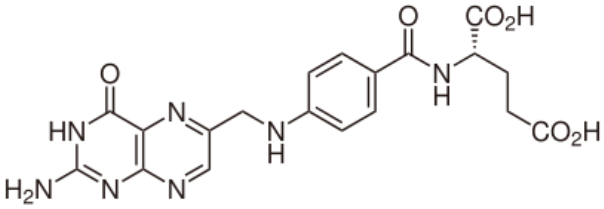
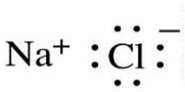
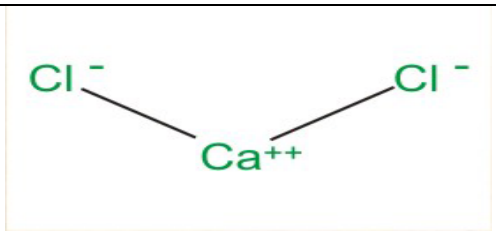
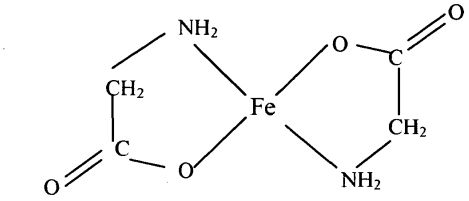
3. Fat: **a. Butter (milk)**	
b. Oil (sunflower)	
4. Vitamins **a. B1 (Thiamine)**	
b. B 6	
c. B9 (Folic acid)	

Table 3.3: Chemical formulas of minerals in food .

5. Minerals : **a. NaCl (Table salt)**	
b. $CaCl_2$ (Calcium Chloride)	
c. Iron Ferrous	

3.2.3.2 **Food Microbes:** The composition of bacterial cell membrane is shown in Figure 3.6

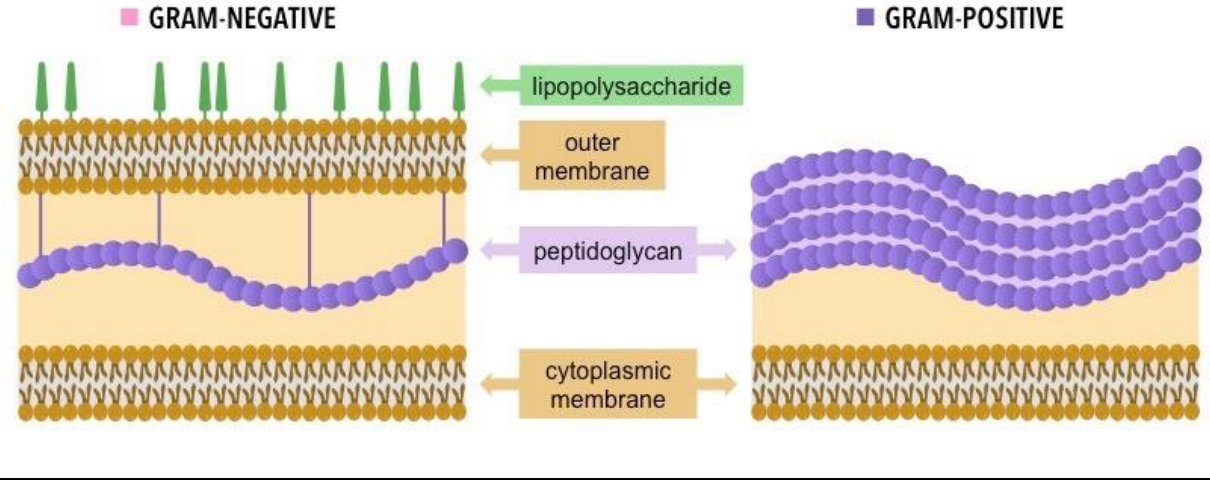
Gram Negatives Bacteria	**Gram Positives Bacteria**
	

Figure 3.6 Cell wall structures of Gram Positives Vs. Gram Negative Bacteria .

3.3. Methods:

Methods applied in this investigation included the build of the set up used in this study, chemical methods in preparing solution of Food Nutrients, and microbiological methods of preparing Food Microbe samples.

3.3.1. Processing Methods:

3.3.1.1. Photopyroelectric Cell Design:

The photopyroelectric cell used in investigation consists of a square piece of biaxially PVDF foil metallised on both sides with aluminum foil to provide for reflecting surfaces and electrode connections. The foil dimension is 1cm ×1cm, 25 μm thickness. The aluminium foil is important to make sure that only heat resulting from radiation absorption and propagating Through the sample is detected and not that generated by direct interaction between radiation and detector. The foil was glued to a block of Perspex glass, which acts as a holder and support for the PVDF. The sample is placed on top of the foil using a micropipette. Perspex block and foil on top constitute the detection cell, it was enclosed in an aluminum box to minimize the ambient electromagnetic interference with the foil, prevent evaporation of the liquid sample during measurements and reduce air turbulence. The infrared radiation was allowed in through a small opening 1cm diameter bored in the aluminium box side. Two electrodes were connected to the top and bottom of the foil with silver paint constituting terminals for the PPE output signal. This cell design was first used by (Ebu-Teir et al., 2008) to study essential oils and olive oil adulteration. Detection cell shown in Figure 3.7 is connected to the electronic monitoring system for signal processing as shown in Figure 3.8.

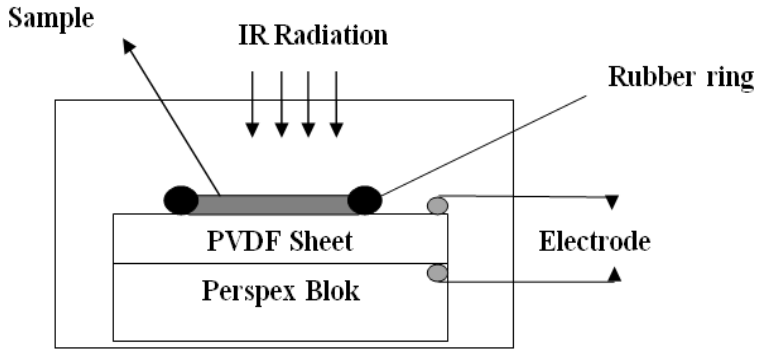


Figure 3.7. Schematic showing the Photopyroelectric cell used to study food sample

3.3.1.2. Wideband infrared (IR) source:

The source is a broadband infrared light source has pulsed operation at large temperature modulations and an active area of 32.7 m^2, rated temperature is 850 C°, minimum and maximum resistances are 2.8 ohms and 4.5 ohms respectively. The source looks dull red at 300 mA, and red at 320 mA, its rated drive power ~ 0.460 watts. It is an electrically pulsed and radiates with low thermal-mass filament tailored for high emissivity in the specified range. This high-efficiency device minimizes drive power, greatly reduces parasitic heating of detectors and optics; it also eliminates the mechanical choppers, permitting a sealed optical path. The high emissivity enables the source to efficiently and rapidly cool via thermal radiation. The hot filament nearly cools to background temperature before the next pulse, thus providing several hundred degrees of temperature modulation. In this study results were obtained at source driving current of 300 mA and modulation frequency of 12.0 Hz.

3.3.1.3. Equipment:

The equipment used in this investigation included three parts, detection units, electronic signal processing system, and IR source. The detection unit is the PVDF and its corresponding cell, either the cell connected to the electric signal processing system, and power function generator. The IR radiation is obtained from pulsed wideband IR source as discussed in previous section 3.3. The source is driven by AC signal from a power function generator; a current of 0.3 Amps is needed to drive the source. The radiation is allowed to fall on the certain amount of food sample placed on the top of PVDF. The absorbed radiation generates a signal that can be picked up from the detection unit, using sensitive detector as shown in figure 3.8.

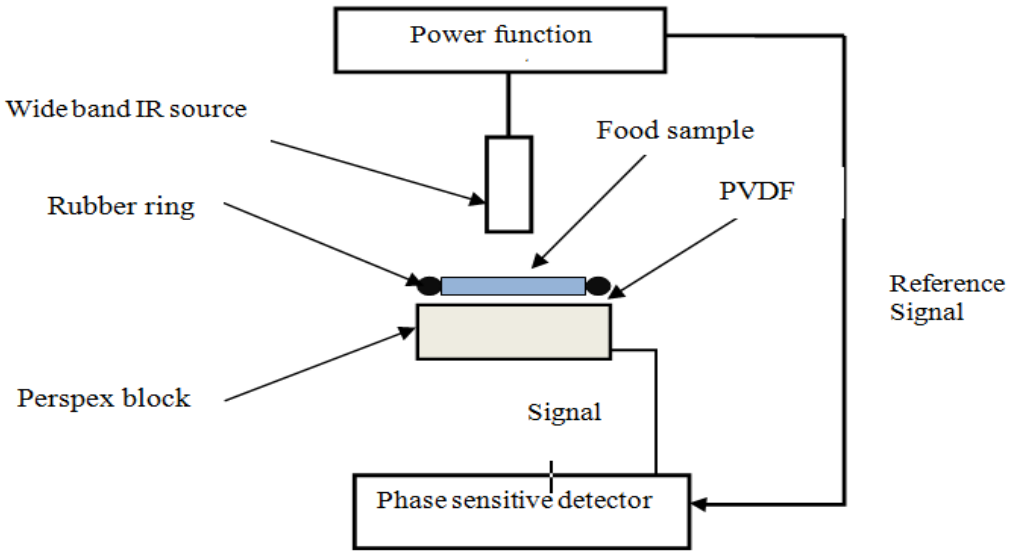


Figure 3.8.Schematic illustration of the complete photopyroelectric detection scheme used to study food samples

3.3.1.4 Experimental Set Up :

The set up used in this investigation was shown in SchematicFigure 3.8

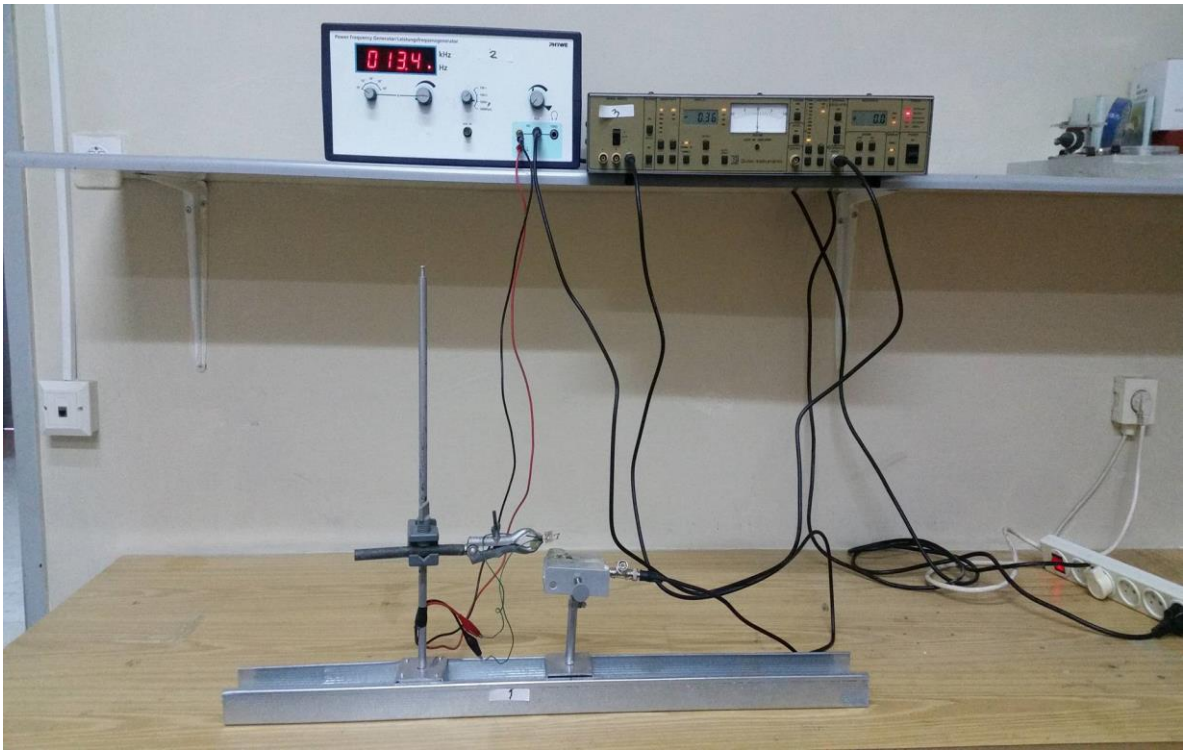


Figure3.9. Photo of complete photopyroelectric setup for food matrix and microbe detection.

3.3.2. Operational Methods:

The investigated sample of food nutrients were pored to the PVD film by dropper in isolated form. Each sample was individually studied and analysed to obtain accurate results

3.3.2.1.Food Nutrients:

There are many ways of expressing concentrations and dilution. All food nutrients sample (sucrose , fat, protein , minerals and vitamins) solutions in this investigation were prepared in different concentrations using the formula: ($C_1V_1 = C_2V_2$) .

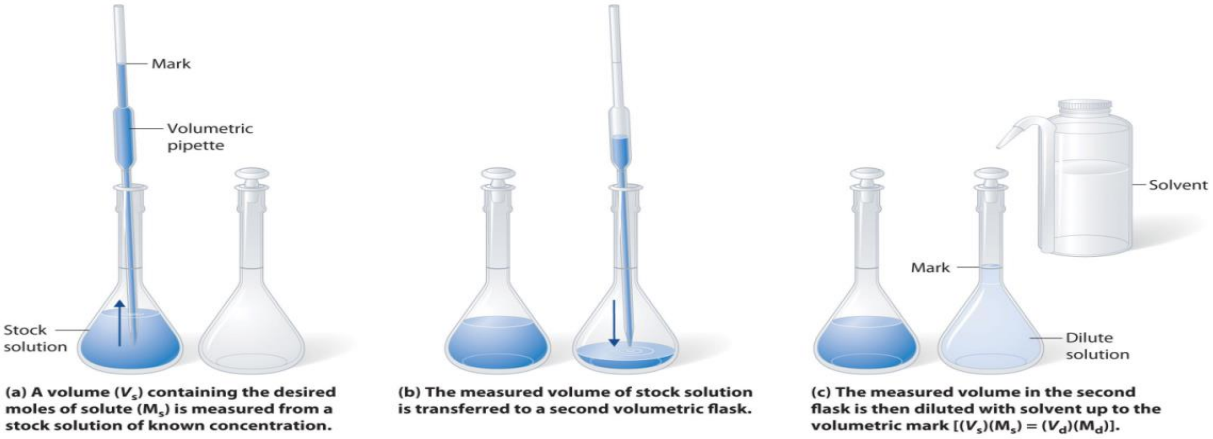


Figure 3.10. Photo shows the solution preparation using formula of ($C_1V_1 = C_2V_2$)

3.3.2.1.1. Food sample preparation:

All these nutrients solution were prepared in (0.1 %, 0.5% , 1% , 1.5 % , 3%, 5% and 10%) concentrations as shown in figure 3.11.

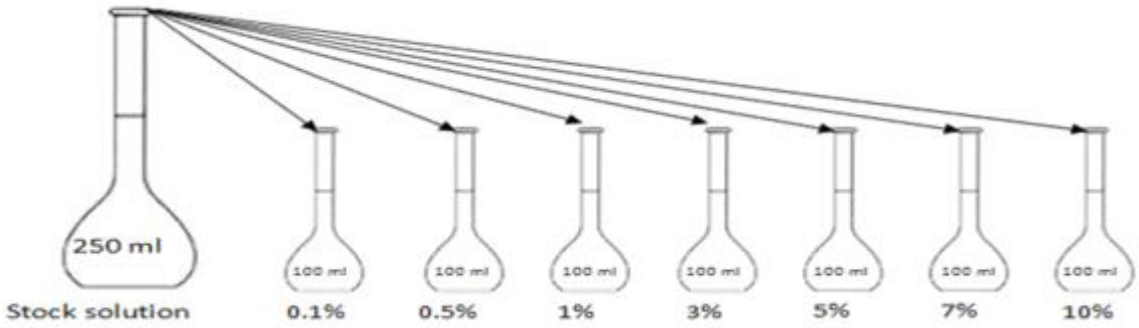


Figure3.11. Photo shows the nutrients solution were preparation.

❖ **Carbohydrate :**

A 25 g pure sucrose was diluted with distilled water in 250 mL flask to prepare the stock solution and then a number of concentrations were formed (0.1 %, 0.5% , 1% , 1.5 % , 3%, 5% and 10%) in volumetric flasks .

❖ **Minerals:**

➢ **NaCl and $CaCl_2$:** Two stock solutions were prepared from 25g of NaCl and $CaCl_2$ by distilled water in 250 ml flask and then the wanted different concentrations were obtained (0.1 %, 0.5% , 1% , 1.5 % , 3%, 5% and 10%) by volumetric flasks.

➢ **Ferrous and Sodium Chloride :** 0.5 g of each mineral were weighed and diluted in 10 mL distilled water to form 5% concentration.

❖ **Protein**:

➢ **Albumin :**

Twenty five grams of albumin crystals diluted in 250 ml flask with distilled water were used to obtained the stock solution which was used to prepare (0.5 %, 1%, 3%, 5%,7%,10% and 12%) by using volumetric flask.

➢ **Amino acids:**

A 5% concentration of four powder samples of threonine, methionine, tryptophan and lysine were formed by weighting 0.5 g of each sample and diluting it with 10 ml distilled water.

❖ **Fat :**

Two different concentrations were prepared (2% and 10%) by weighting 2 g and 10g of sunflower oil and milk butter and diluted in 100 volumetric flask with chloroform.

❖ **Vitamins:**

A small amounts **(**0.5 g) of each vitamins B1 (thiamine), B6 and B9 (folic acid) were used with distilled water in 10 mL flask for each one to prepare a dilution of 5% concentration for study.

❖ **Real food sample:**

Milk:

Under aseptic conditions, a drop of 100 µL of three different types of milk were studied: raw milk, pasteurized milk, and UHT milk. Each types of milk was studied in two different conditions, under ambient temperature and under refrigeration. All samples were studied through different storage period of time (after 1 day, 2 days, 3days, 7 days, and 10 days) as seen in figure 3.12.

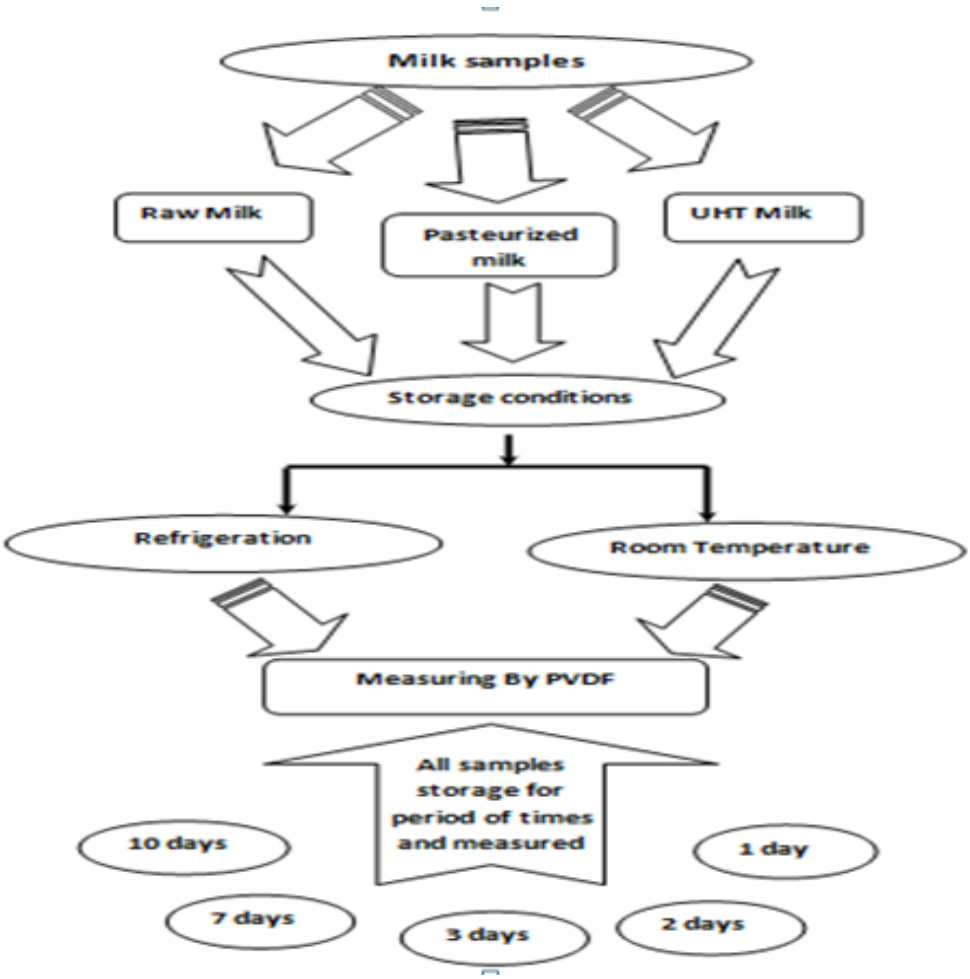


Figure 3.12. Flowchart of Milk samples preparation for study.

Egg:

A drop (100µL) of egg yolk, egg white and whole egg were studied using PVDF film separately under aseptic conditions as shown in figure 3.13.

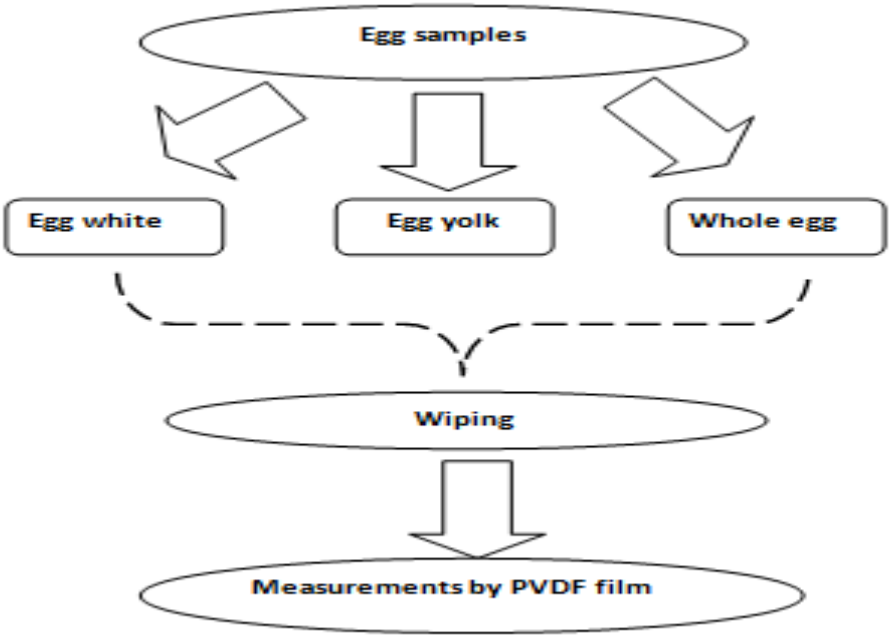


Figure 3.13. Flowchart showed the Egg sample preparation for measurement by PVDF film.

3.3.2.2. Food Microbes Analysis:

Isolated samples of *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*, yeast(*Candida*) and Mold samples were prepared by using standard method of microbial testing as Bacteriological Analytical Manual (BAM) which presents the agency's preferred laboratory procedures for microbiological analyses of foods; at biology lab in Al-Quds University. A selective (specific) growth media was used for individual microbes used in this study: Brilliant Green Agar (BGA) for *Salmonella*, Eoisin Methylene Blue (E.M.B) Agar for *E.Coli*, Baird Parker Medium for *S.aureus* and O.E.Y.E Agar for Yeast and Mold.

.

Three different dilutions($1\times10^{\wedge-1}$, $1\times10^{\wedge-2}$, $1\times10^{\wedge-3}$) of each microbe sample were formed in 9 mL of peptone water and other 9mL in distilled water. All samples pored to PVDF for

detection by micropipette (100 µL) and all aseptic condition was take in to consideration during the run experiments and all sample saved in refrigerator during bacterial detections.

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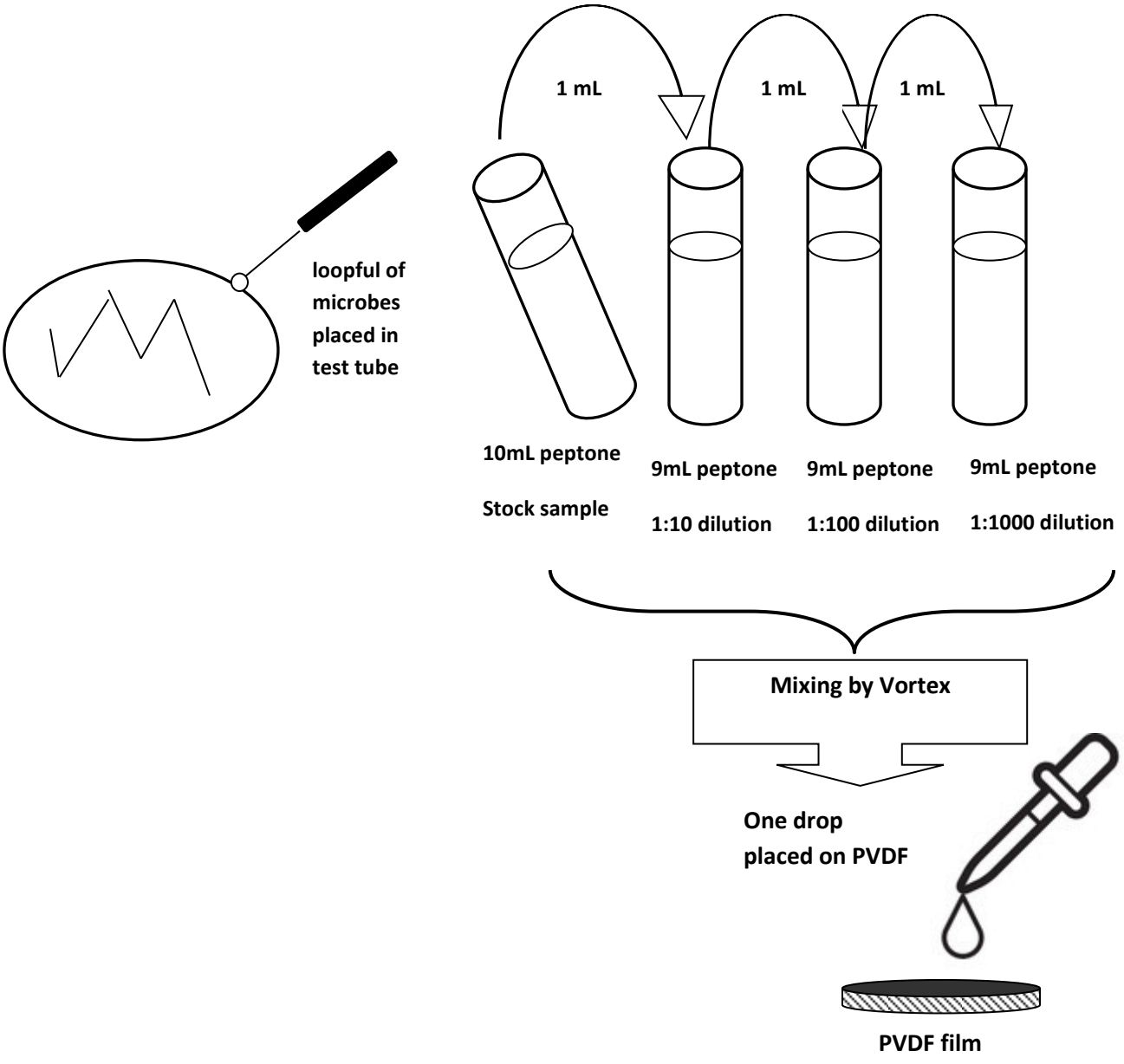


Figure 3.14. Schematic illustration of food microbes dilutions prepared for measurements by PVDF film.

3.3.3. Detection Method:

Different sample of food nutrients and microbes were investigated to study its reaction with ability of PVDF film to react with the studied nutrients .In each run a single drop of 10 µL by micro pipette was placed on the surface of PVD film in the region of ring rubber. The radiation of IR was driven from the power function generator and fallen directly to the sample, where each sample react with absorbed radiation depends on its structure and functional group, and it's resulting absorption of IR radiation by food or microbe sample generates a signal that is picked up by the detection system and recorded as shown in figure 3.15

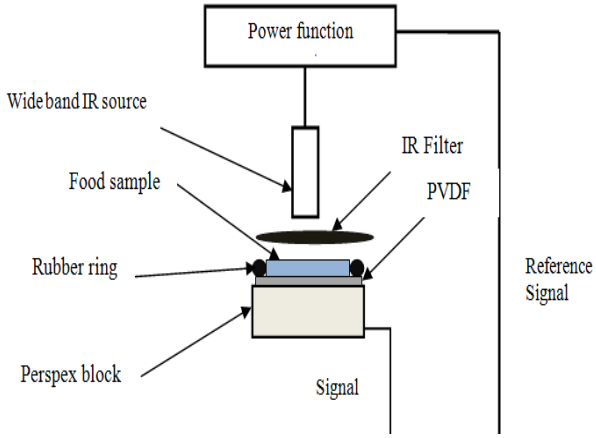


Figure 3.15. Schematic illustration of the complete photopyroelectric detection scheme used to study food samples with filters

Each sample examined was repeated three times to reduce the analytical mistakes. The process was performed under aseptic condition during sample tested as shown in section 3.3.

3.3.3.1. Cleaning the Cell:

After each run used the PVDF film was cleaned carefully by swap distilled water and after microbial testing after each microbe the PVDF film carefully cleaned by 97% alcohol and distilled water to ensure that all microbes are killed.

.

3.3.4. IR Filters:

IR filers in different wave lengths (720nm, 760nm, 850nm, 950nm, 1000nm) were invested to examine the experimental sample to define the specific food matrix and microbes. In this study five filters were used to investigate the ability of this technique to detect each food component and differentiate it from other components or materials. All samples studied by each filter separately, these filters were placed over the PVDF film and directly under the IR source, to allow a specific wavelength to pass throw. As shown in figure 3.15.

3.3.5 Quality Assurance Test:

This investigation is based on a fact that each food element of constituents should have one specific wavelength to be measured by. Enable to verify this assumption one measure quality assurance test was conducted to validate this assumption.

The test based on that each filter will be specified for each food elements. Thus as the results showed, five major nutrients and food elements were linked with specific wavelength through IR-filters. One of these five food nutrients was chosen to be measured with its specific IR-filter and to compare its results with measuring it again by using two filter at the same time.

3.3.6 Methodology:

The goal of this study is to introduce a new technique for food analysis, Analysis of foods is continuously requesting the development of more robust, efficient, sensitive, and cost-effective analytical methodologies to guarantee the safety, quality, and traceability of foods in compliance with legislation and consumers' demands.

All samples that are prepared in different concentrations were placed under the IR source and each sample showed a remarkable signal that picked up by the detector will go further experiment using IR filters.

Sample which generates signals will be compared with other sample to catch if there are any two different nutrients that release the same reading with the same filter.

4.1 Introduction:

This chapter shows the results obtained and introduces the required discussion for the results which will introduce better understanding for the mechanism and the goal and purpose of this investigation. The results introduced are classified according to types of measurements, namely: measurement at full beam of infrared radiations, measurement at certain infra-red band by using Infrared wideband pass filters, and measurement for real food sample.

4.2 Measurements at Full Beam IR Source:

As seen in Tables 4.1,4.2, and 4.3 all used samples absorbed the broad band infrared light radiation, while it on the top of the PVDF film shown an noticeable different reading with the PVDF film depends on types of components, e.g. the signals that obtained from Sucrose at different concentrations completely differed from that obtained from albumin, which could be defined due to the differences in food components and microbes structures and their functional groups(C-H, COOH, N-H, C=O) and its reaction with the pohtopyroelectric film (PVDF), the signals obtained from samples differed due to the difference in their chemical structures, when IR radiations hit the molecules, the radiation induces a vibrational excitation of the covalent bonds and groups, this excitation causes the bond to stretch or bent.

The chemical bonds have a specific frequency and it vibrates due to exposure to IR radiations, this frequency is related to the strength of bond or the mass of the atoms on the end of the molecules or its functional group.

The amount of the absorbed radiations is related to the strength of chemical bond, the different kinds of chemical bond in food materials like: (carbonyl group C=O, carboxyl group COOH, hydroxyl group OH, amine N-H, methyl group, double and triple bond C=C) induced different vibration.

Table 4.1: Values of different samples at different concentrations measured by full beam of wideband infrared source.

Type of samples:	Different concentrations (%)						
	0.1%	0.5%	1%	3%	5%	7%	10%
Sucrose	0.5	0.7	0.9	1.3	1.3	5.6	5.4
Albumins	6	6.1	7.2	6.2	6.9	9.7	5.3
NaCl	0.5	2.7	0.9	1.1	6.3	8.3	7.8
$CaCl_2$	4.5	3.6	3.7	2.5	3.7	4.0	4.1

As noticed in Table 4.1 signal readings of same sample were vary, as different concentration were investigated. The concentrations were started from 0.1% and increasing to 10% for each material tested. For example the sucrose generates signals increased from 0.5 to 5.4 with increasing the concentrations.

Same finding noticed in the signals that generates from table salt and range from 0.5 to 7.8, however the signals that obtained from calcium chloride slightly more than sucrose and table salt at 0.1% concentration was 4.5 while its 0.5 in both sucrose and salt.

In the other hand signals obtained from protein (Albumin) has more reactivity with the PVDF at concentration 0.1% and decreased with increasing in concentrations, it's obvious that at concentrations 3% all signals at all sample had other pathway which all exhibit a decrease in signals measurement and retained to increased again for higher concentrations, the possible explanation for such case could be due to the reaction of PVDF with the variation in the sample structure and properties like the presence of the hydroxyl group in sucrose(OH), amine group in protein (NH), and ionic properties of both NaCl, $CaCl_2$ react with PVDF in differently.

As seen in Table 4.2 all sample react with PVDF in different manor. Unlike to the result of reacting PVDF with previous sample (sucrose, protein, NaCl, $CaCl_2$) were the albumin had the highest reading but as seen in Table 4.2 the all sample at 5% concentration of vitamins, fat, and minerals like ferrous have high signal reading while the amino acid had shown a lower signal output in an exception for tryptophan which had a higher reading like other samples. Most sample generates signals around 5 (a.u) while, vitamin B_6generated around 8. This result could be explained due to sample chemical structure and their different functional group and behavior at water and its ability to IR absorption.

Table 4.2 Values of different samples at 5% concentrations measured by full beam of wideband infrared source

Type of samples	Signals values at concentrations (5%)
amino acids	
Tryptophan	5.5
Thrionine	0.58
Methionine	0.64
Lysine	0.74
Fat	
sunflower oil	5.53
Butter	5.3
Vitamins	
B_1	6.72
B_6	8.02
B_9	5.92
Minerals:	
Ferrous	6.65
Ultra Pure Sodium chloride	5.82

As noticed in Table 4.3 the reactivity of food microbes namely: *E.Coli, salmonella, pseudomonas, S.aureus, Bacillus, listeria, mold* and *yeast (Candida)* with PVDF film generate different signals by different microbes, in this Table results showed that all microbes belonges to Negative Gram bacteria (*E.coli, Salmonella, Pseudomonas*) have higher values around 6.91, 8.97, 3(a.u) respectively, compared to the values of Positive Gram bacteria (*S.aureus, Bacillus, Listeria)* that have lower values around 0.68, 0.23, 1.05 (a.u), respectively. However this results could be assumed to the difference between Gram Negative and Gram positive bacteria structure in their cell wall in presence of thick peptidoglycan layer in Gram Negative whereas absence in the positive gram bacteria.

Table 4.3 Values of food micro-organisms samples measured by full beam of wideband infrared source.

Micro-organism sample	**(CFU)Diluted in one drop of distilled water**
E.Coli (-ve)	6.91
S.aureus (+ve)	0.68
Salmonella (-ve)	8.97
Pseudomonas (-ve)	3.01
Bacillus (+ve)	0.23
Listeria(+ve)	1.05
Mold	0.47
Candida	3

On the other side, the activity of mold and yeast with PVDF film different as seen in the Table 4.3 the yeast *candida* generates more signals than mold,3 and 0.47 respectively which

depends on the difference in their structure :molds are microorganisms which have a tendency to grow with multiple cell filaments called hyphae, whereas yeast is a microscopic form of fungi having just a one cell.

4.3 Measurements at Certain Wavelength:

Measurement of repaired samples were took place by using five different IR-filters that allow only one wavelength to be reacted with the food sample and PVDF film. Since the investigated food nutrients, real food sample, and microorganisms had showed high interaction with the full beam wavelength of infra-red and the PVDF. Thus certain nutrients and microbes were reacted with the specific wavelength and the reset need another wavelength to be linked with.

4.3.1Measurements at Available Specific IR Wavelength:

4.3.1.1 Measurement of NaCl:

As shown in Table 4.4, NaCl solutions with different concentration showed a reasonable readying with full beam spectrum of IR. Using the five available filters showed non reacted activity between NaCl samples and filters with certain wavelength, namely; 1000, 950, 850, and 760. At the same time the NaCl solutions expressed a noticeable measuring in the absorbance figures measured under the use of 720*nm* bandpass IR-filter.

Table 4.4 The values of different concentration of NaCl solution measured by different IR wavelength.

Concentration (%)	Absorbance under Full beam wavelength	Absorbance With filter At specific wavelength				
		1000	950	850	760	720
0.5%	2.74	2.743	2.69	2.69	2.5	2.6
1%	0.9	0.8175	0.8025	1.08	2.184	0.592
3%	1.12	0.936	0.718	0.522	0.648	0.883
5%	6.29	0.14	1.366	1.303	1.06	1.19
7%	8.28	1.61	1.136	0.81	0.855	1.23
10%	7.8	1.154	1.346	0.7366	0.684	2.228

At the same time, measurements of the NaCl concentrations under the 720 *nm* IR filter showed certain trend, as the concentration of NaCl increased the absorbance value increased, as appeared in Figure 4.1. However, the results trend appeared to be in linear mode with R^2 =0.91.

This result due to the reaction between PVDF film and NaCl which found to react positively. The possible explanation for such reaction is the ability of PVDF porosity to react more with more NaCl molecules presents.

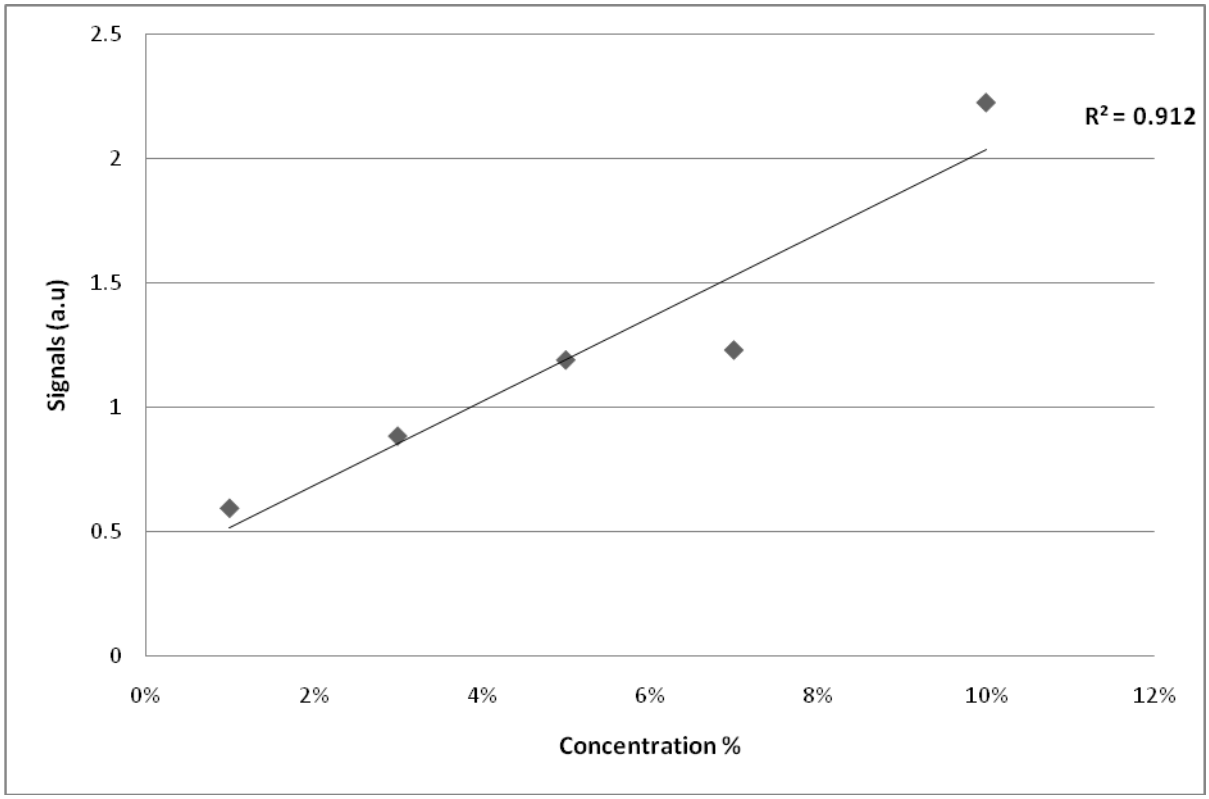


Figure 4.1 the trend of different NaCl concentration measured under 720 nm IR wavelength.

4.3.1.2 Measurement of $CaCl_2$:

In addition, as shown in Table 4.5, a reasonable readying with full beam spectrum of IR were obtained from $CaCl_2$ solutions with different concentration. Using the five available filters showed non reacted activity between $CaCl_2$ samples and filters with certain wavelength, namely; 1000, 950, 850, and 760. At the same time $CaCl_2$ solutions were expressed a noticeable measuring in the absorbance figures measured under the use of 850nm IR-filter.

Table 4.5 The values of different concentration of $CaCl_2$ solution measured by different IR wavelength

Concentration (%)	Full beam	With filter (nm)				
		1000	950	850	760	720
0.5%	4.5	0.17	0.17	1.233	0.283	0.123
1%	3.6	0.11	0.477	1.443	0.46	0.417
3%	3.7	0.443	0.443	1.47	0.453	0.443
5%	2.5	0.25	0.187	1. 567	0.15	0.233
7%	3.7	0.48	0.383	1.57	0.367	0.27
10%	4.0	0.507	0.127	1.49	0.553	0.623

As well as, measurements of the $CaCl_2$ concentrations under the 850nm IR filter displayed certain trend, as the concentration of $CaCl_2$ increased the absorbance value increased, as appeared in Figure 4.1. However, the results trend appeared to be in linear mode were R^2 was equal to 0.89.

This result due to the reaction between PVDF film and $CaCl_2$ found to be positive reaction. The possible explanation for such reaction is the ability of PVDF porosity to react more with more $CaCl_2$ molecules presents.

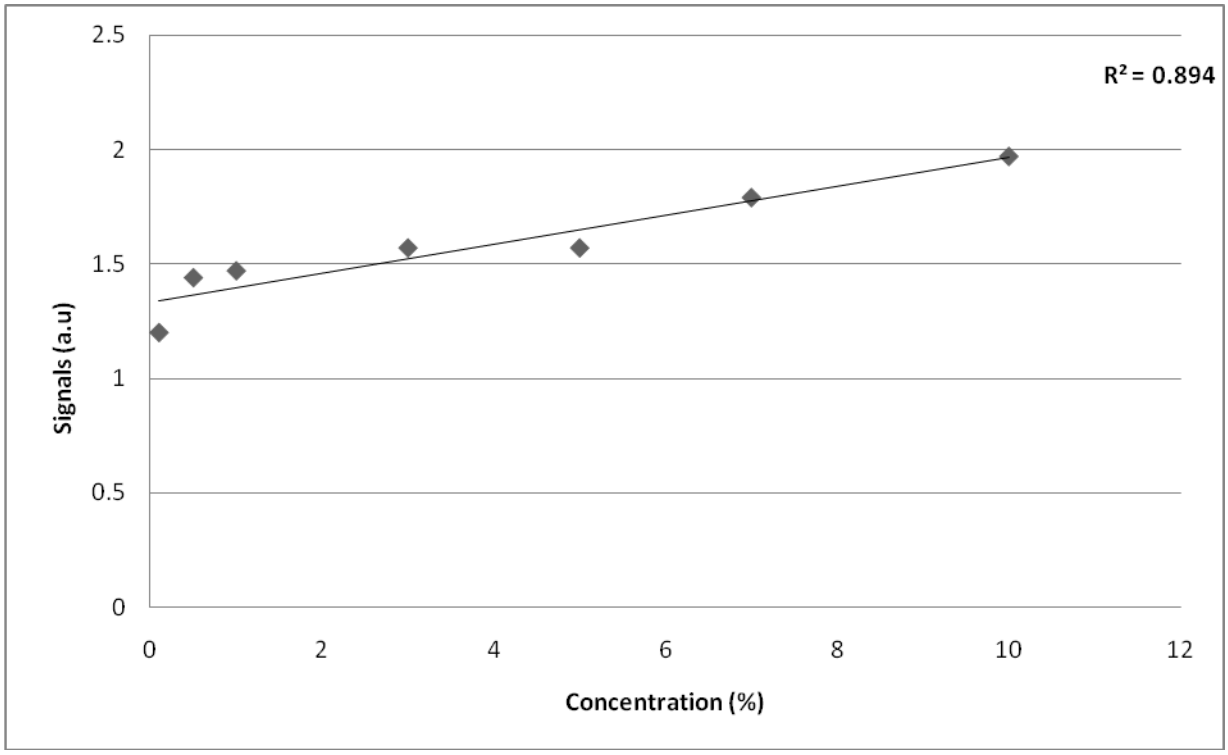


Figure 4.2 Trend of different $CaCl_2$ concentration measured under 850 nm IR wavelength.

4.3.1.3 Measurement of Sucrose:

In the same manner, as shown in Table 4.6, Sucrose solutions with different concentration presented a reasonable readying with full beam spectrum of IR. Using the five available filters showed non reacted activity between Sucrose samples and filters with certain wavelength, namely; 1000, 950, 850, and 760. At the same time the Sucrose solutions expressed a noticeable measuring in the absorbance figures measured under the use of 1000 nm IR-filter.

Moreover, measurements of the sucrose concentrations under the 1000nm IR-filter showed certain trend, as the concentration of sucrose increased the absorbance value increased, as appeared in Figure 4.3. However, the results trend appeared to be in logarithmic mode which R^2=0 .978.

This result due to the reaction between PVDF film and sucrose found to be positive reaction. The possible explanation for such reaction is the ability of PVDF porosity to react more with more sucrose molecules presents.

Table 4.6 The values of different concentration of Sucrose solution measured by different IR wavelength

Concentration (%)	Full beam	With filter (nm)				
		1000	950	850	760	720
0.5%	0.48	3.308	0.254	0.483	0.3	0.205
1%	0.66	3.355	0.426	0.873	1.048	0.845
3%	8.5	3.39	1.07	1.034	0.672	1.023
5%	1.25	3.332	1.24	1.478	1.178	1.134
7%	1.3	3.323	1.055	1.536	1.482	1.465
10%	5.56	3.49	1.415	1.592	1.566	1.702

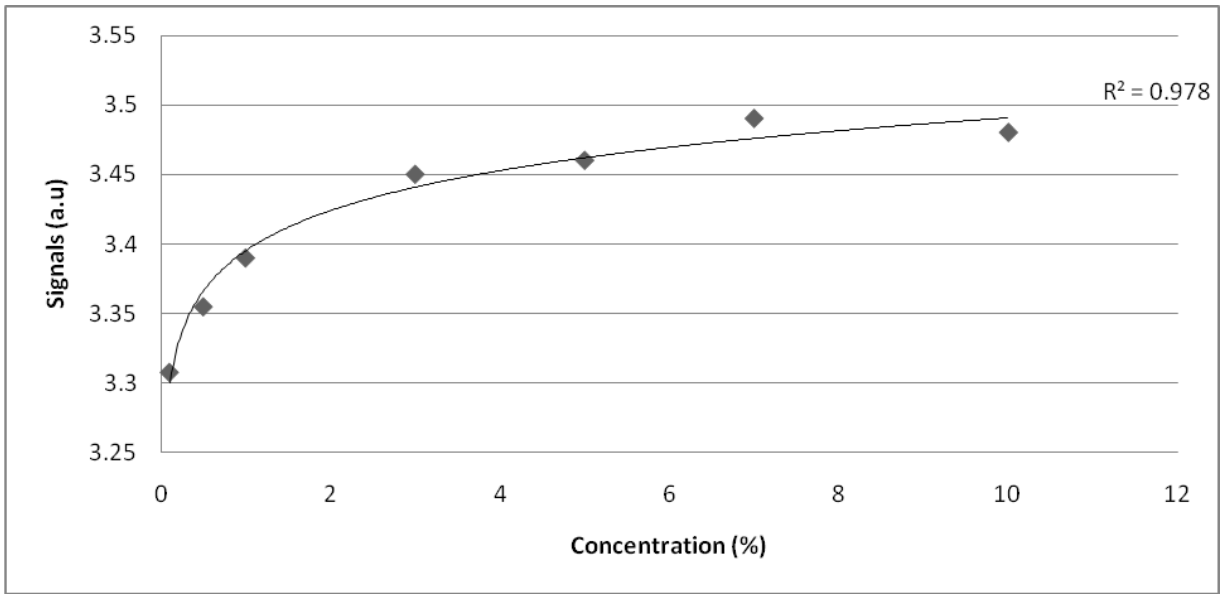


Figure 4.3 Trend of different sucrose concentration measured under 1000nm IR wavelength.

4.3.1.4 Measurement of Tryptophan:

Furthermore, as shown in Table 4.7, tryptophan solutions at 5% concentration presented a reasonable readying with full beam spectrum of IR. Using the five available filters showed non reacted activity between Sucrose samples and filters with certain wavelength, namely; 1000, 950, 850, and 760. At the same time the tryptophan solutions expressed a noticeable measuring in the absorbance figures measured under the use of 760 nm IR-filter.

Table 4.7. The values of tryptophan at 5% concentration solution measured by different IR wavelength.

Concentration (%)	Full beam	With filter (nm)				
		1000	950	850	760	720
5%	5.541	1.1	1.3	2.4	5.5	2.4

Unfortunately, due to the lack of tryptophan sample, no further investigation in term of a seires of different concentrations were took place.

4.3.1.5 Measurement of Candida:

As seen in Table 4.8, a reasonable readying with full beam spectrum of IR were obtained for *Candida* dilution. Using the five available filters showed non reacted activity between *Candida* samples and filters with certain wavelength, namely; 1000, 950, 850, and 760. At the same time the *Candida* dilution showed a noticeable measured readings in the absorbance figures measured under the use of 950 nm IR-filter.

Table 4.8. The values of candida stock concentration measured by different IR wavelength.

Concentration (%)	Full beam	With filter (nm)				
		1000	950	850	760	720
5%	4	1.2	3.1	1.7	1.6	1.34

This result was confirmed when a series of different dilution were prepared for candida and the signal measurement was took place by using 950nm IR wavelength, as plotted in Table 4.9.

The measurements obtained showed a gradual decrease in absorbance measurement value as the dilution increased. This due to the fact that the PVDF film was able to react with higher number of candida cells more than with lower number of candida cells, thus more cells produces higher reading value.

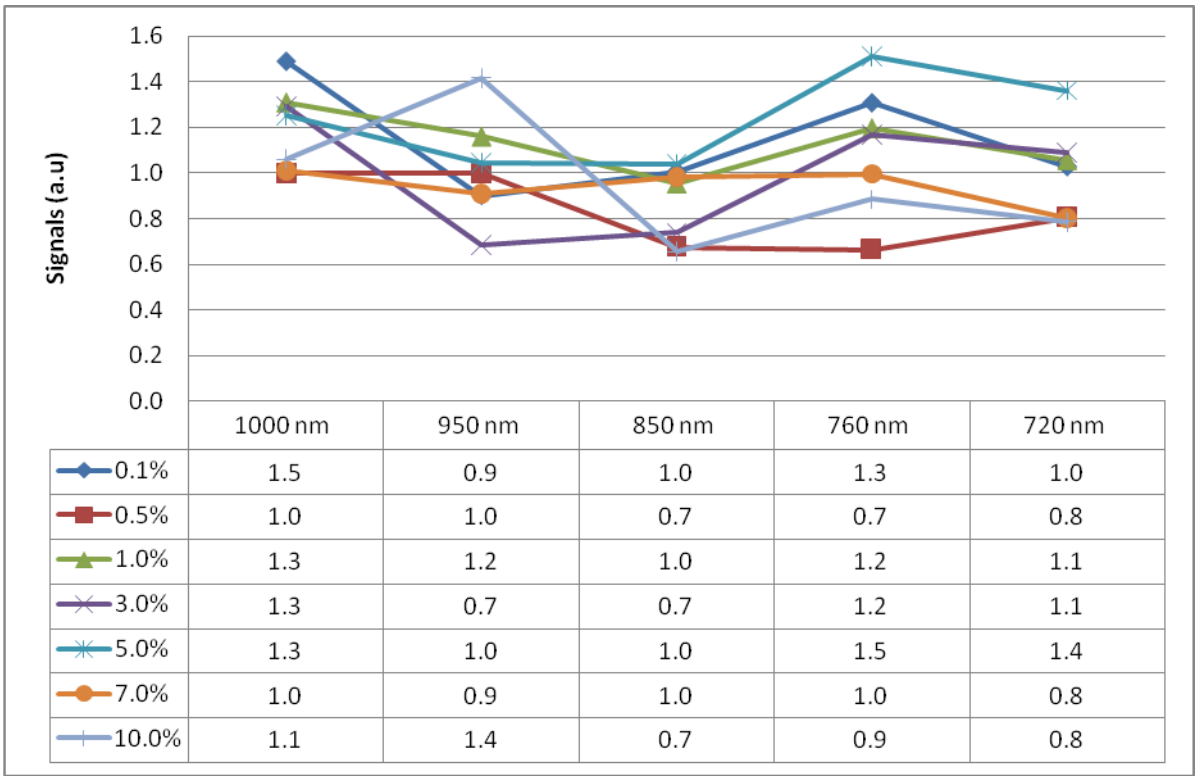
Table 4.9. The values of candida different dilution measured by 950nm IR wavelength.

No.	Candida dilution	signal measurement (a.u)
1	1	4.8
2	0.1	4
3	0.01	3.7
4	0.001	3.4
5	0.0001	3.1
6	0.00001	2.1
7	0.000001	2.2

4.3.2Measurements at Unavailable Specific IR Wavelength:

In contrast to previous results, in this section all samples showed only a noticeable reaction with full beam of IR but no obvious reaction with certain IR-filters, the possible assumption for such results is that sample could react with other filter with certain wavelength rather than the available one in this study, which the budgets of this study couldn't possible to achieve all filters set that cover infrared radiation.

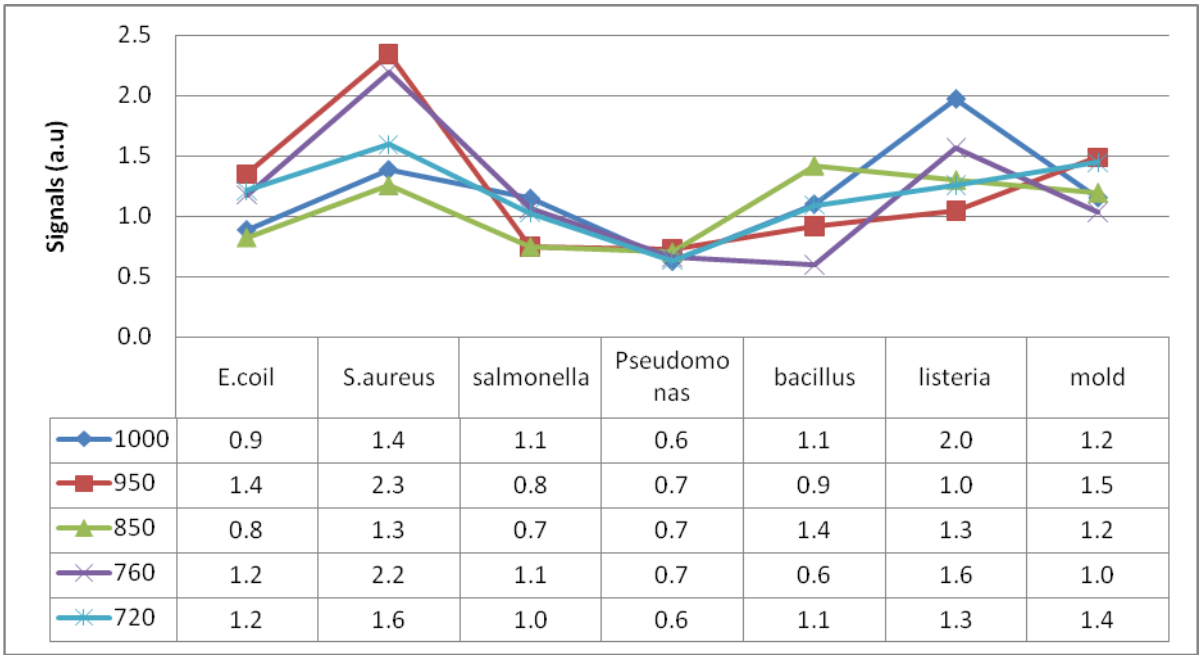
As examined in Figure 4.4, all albumin samples showed no reaction with the available filters with certain wavelength, namely; 1000, 950, 850,760 and 720 nm.



	1000 nm	950 nm	850 nm	760 nm	720 nm
0.1%	1.5	0.9	1.0	1.3	1.0
0.5%	1.0	1.0	0.7	0.7	0.8
1.0%	1.3	1.2	1.0	1.2	1.1
3.0%	1.3	0.7	0.7	1.2	1.1
5.0%	1.3	1.0	1.0	1.5	1.4
7.0%	1.0	0.9	1.0	1.0	0.8
10.0%	1.1	1.4	0.7	0.9	0.8

Figure 4.4 Values of IR-filters with different albumin concentrations.

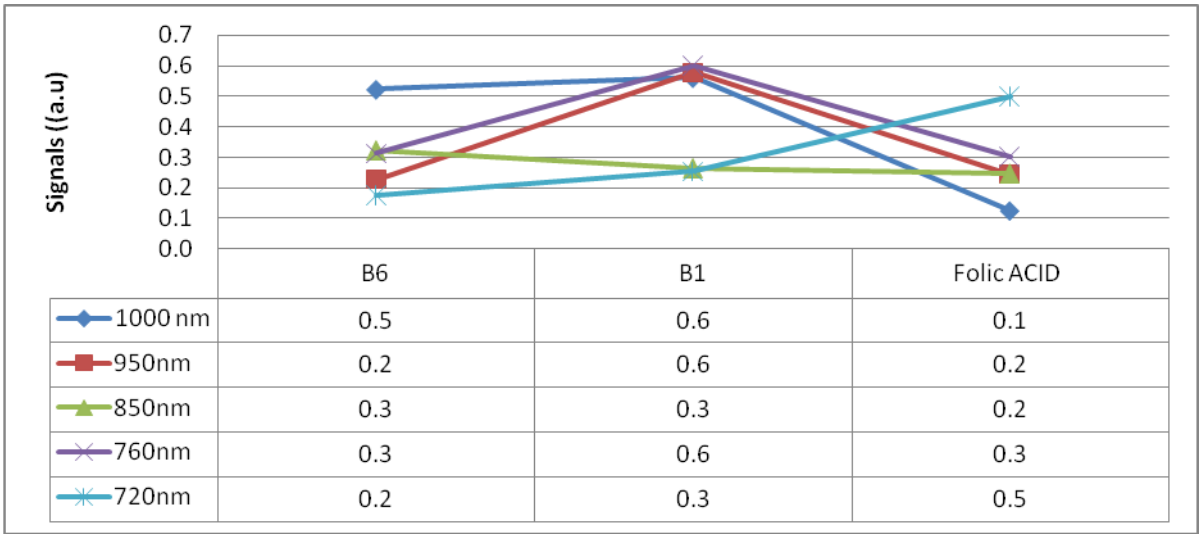
Same finding was obtained for different types of microbes, as shown in Figure 4.5. All investigated microbes were able to be measured with full beam IR wavelength, but the available IR-filters were unsuitable for specific microbes.



	E.coil	S.aureus	salmonella	Pseudomonas	bacillus	listeria	mold
1000	0.9	1.4	1.1	0.6	1.1	2.0	1.2
950	1.4	2.3	0.8	0.7	0.9	1.0	1.5
850	0.8	1.3	0.7	0.7	1.4	1.3	1.2
760	1.2	2.2	1.1	0.7	0.6	1.6	1.0
720	1.2	1.6	1.0	0.6	1.1	1.3	1.4

Figure 4.5 Values of IR-filters with different food microbes.

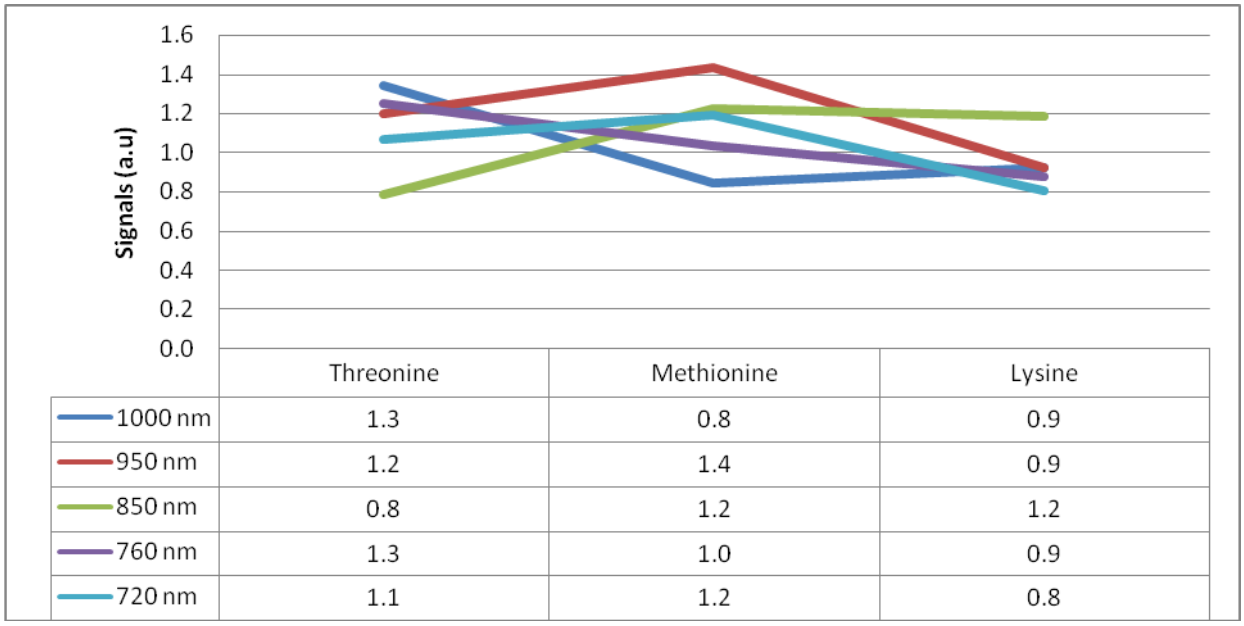
Furthermore, same obstacles were found in measuring vitamins by available IR filters. The investigated vitamins were subjected for full beam of infra-red showed a reasonable measurements. At the same time the available IR-filters were unsuitable for specific wavelength readings, as appeared in Figure 4.6.



	B6	B1	Folic ACID
1000 nm	0.5	0.6	0.1
950nm	0.2	0.6	0.2
850nm	0.3	0.3	0.2
760nm	0.3	0.6	0.3
720nm	0.2	0.3	0.5

Figure 4.6 Values of IR-filters with vitamins samples.

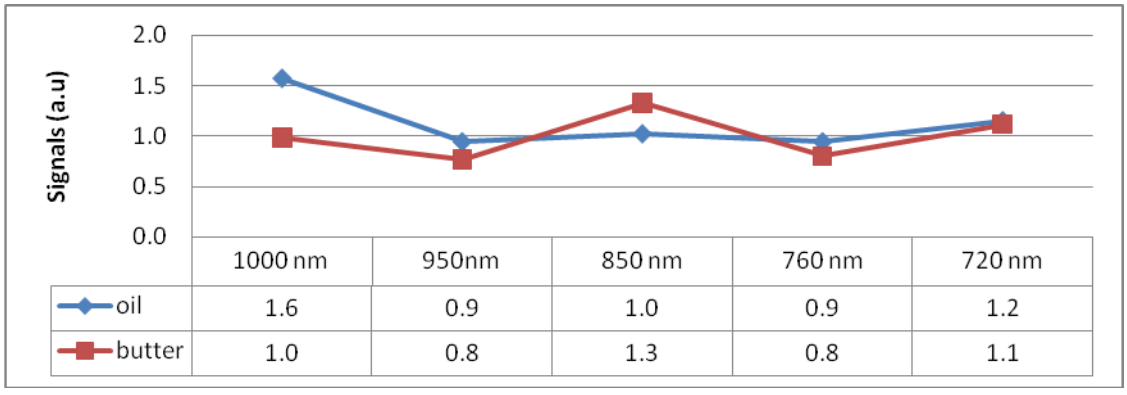
Although amino acids involved in this investigation, namely; Threonine, Methionine, and Lysine showed a measurable value under the effect of full beam infra-red source. At the same time non one of the available IR-filters showed constant reading for the amino acids samples tested (Figure 4.7).



	Threonine	Methionine	Lysine
1000 nm	1.3	0.8	0.9
950 nm	1.2	1.4	0.9
850 nm	0.8	1.2	1.2
760 nm	1.3	1.0	0.9
720 nm	1.1	1.2	0.8

Figure 4.7 Values of IR-filters with amino acids.

The last sample investigated was the lipid sample. Samples of oil and butter were tested under the full beam wavelength of IR source. While these samples showed an interaction with the PVDF and exhibit a valuable reading, the available specific IR wave length showed no reading.



	1000 nm	950nm	850 nm	760 nm	720 nm
oil	1.6	0.9	1.0	0.9	1.2
butter	1.0	0.8	1.3	0.8	1.1

Figure 4.8 Values of IR-filters of lipid materials.

Results obtained in this section was shown in Figure 4.8. The investigation emphasize on the ability of the PVDF to interact with those materials and managed to provide certain measurement of considerable values when the full beam of the IR wavelength were used. This means that those materials need a specific wavelength rather than the available ones. As non-one of the available five IR-filters was suitable for those materials.

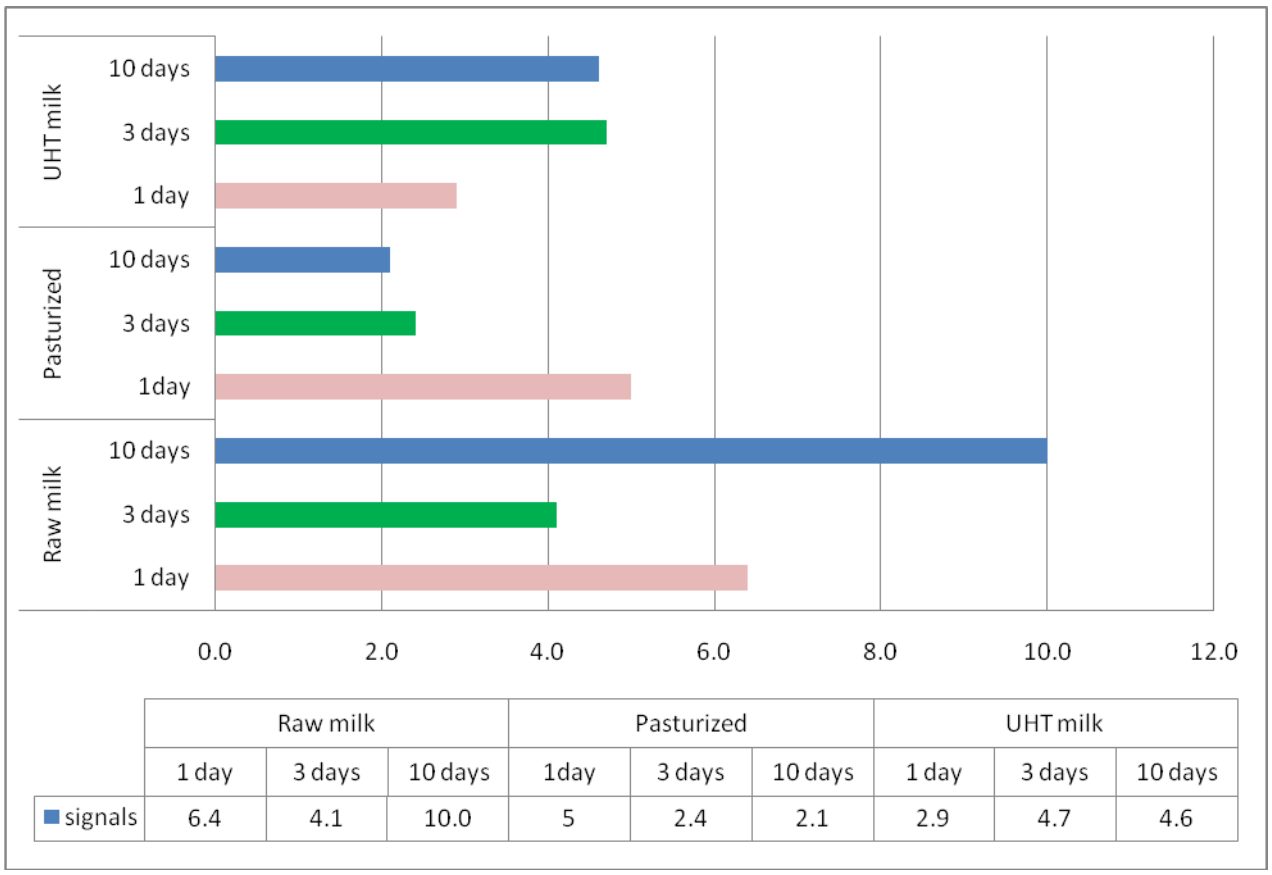
4.4 Real Sample Measurements with PVDF Film:

After examined food single items and microbes on PVDF film to study each of them separately and under certain IR wavelength, different types of real food samples were used, namely: Raw milk, pasteurized milk, UHT milk, whole egg, egg white, and egg yolk individually at certain storage conditions.

4.4.1 Milk Samples:

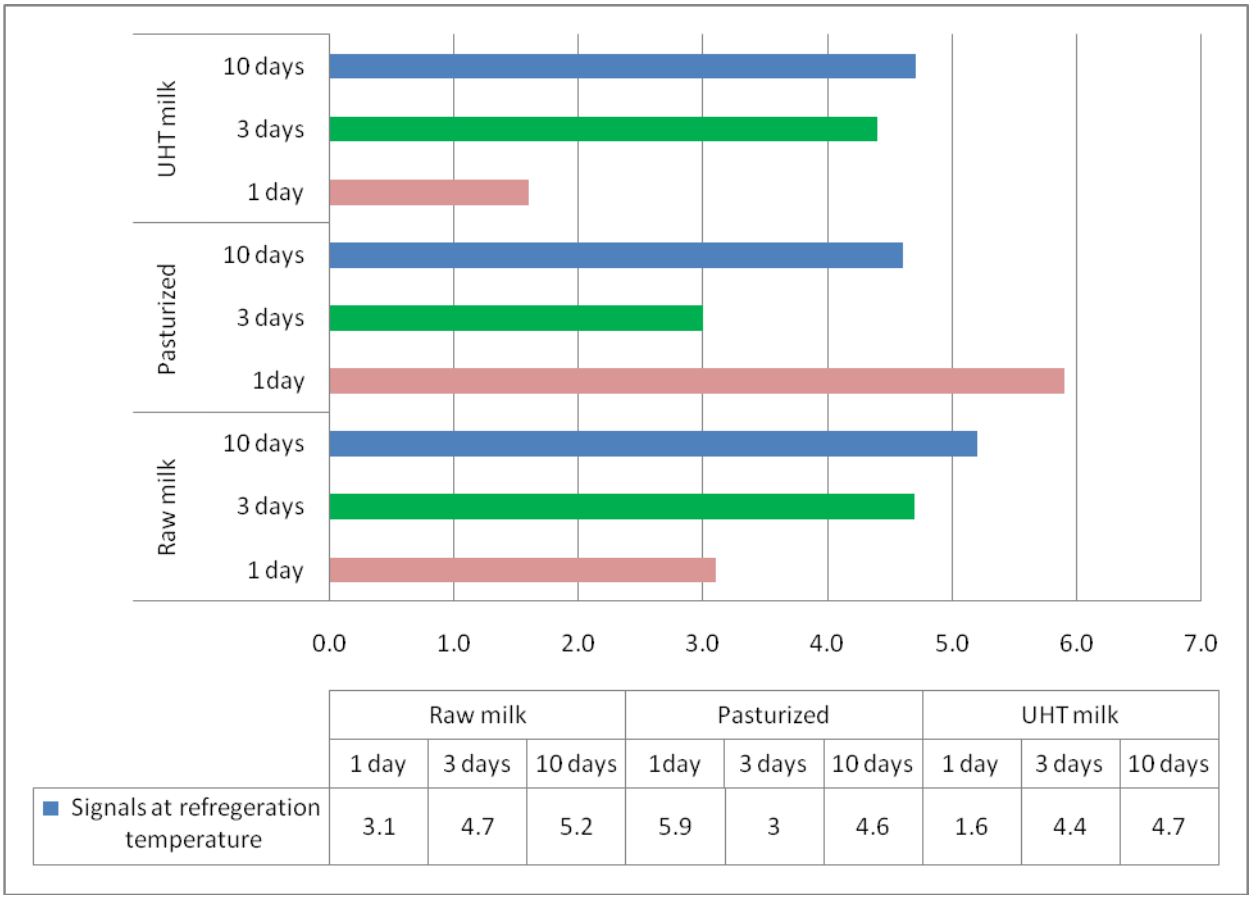
Studying the obtained results showed a remarkable variation between milk samples stored at room temperature. The highest measuring value was to raw milk samples. This is due to the ability of raw milk to develop higher number of microbial growth in comparison to pasteurized and sterilized (UHT) milk samples.

Same trend with lower magnification were obtained for milk samples stored at refrigeration temperature. This logical and expected results due to the highly effect of storage temperature on microbial growth, as shown in Figure 4.9 and Figure 4.10 for storage at room temperature and refrigerated temperature, respectively.



	Raw milk			Pasturized			UHT milk		
	1 day	3 days	10 days	1day	3 days	10 days	1 day	3 days	10 days
signals	6.4	4.1	10.0	5	2.4	2.1	2.9	4.7	4.6

Figure 4.9 Values of milk types with PVDF at room temperature storage.



	Raw milk			Pasturized			UHT milk		
	1 day	3 days	10 days	1day	3 days	10 days	1 day	3 days	10 days
Signals at refregeration temperature	3.1	4.7	5.2	5.9	3	4.6	1.6	4.4	4.7

Figure 4.10 Values of milks with PVDF under refrigerator storage.

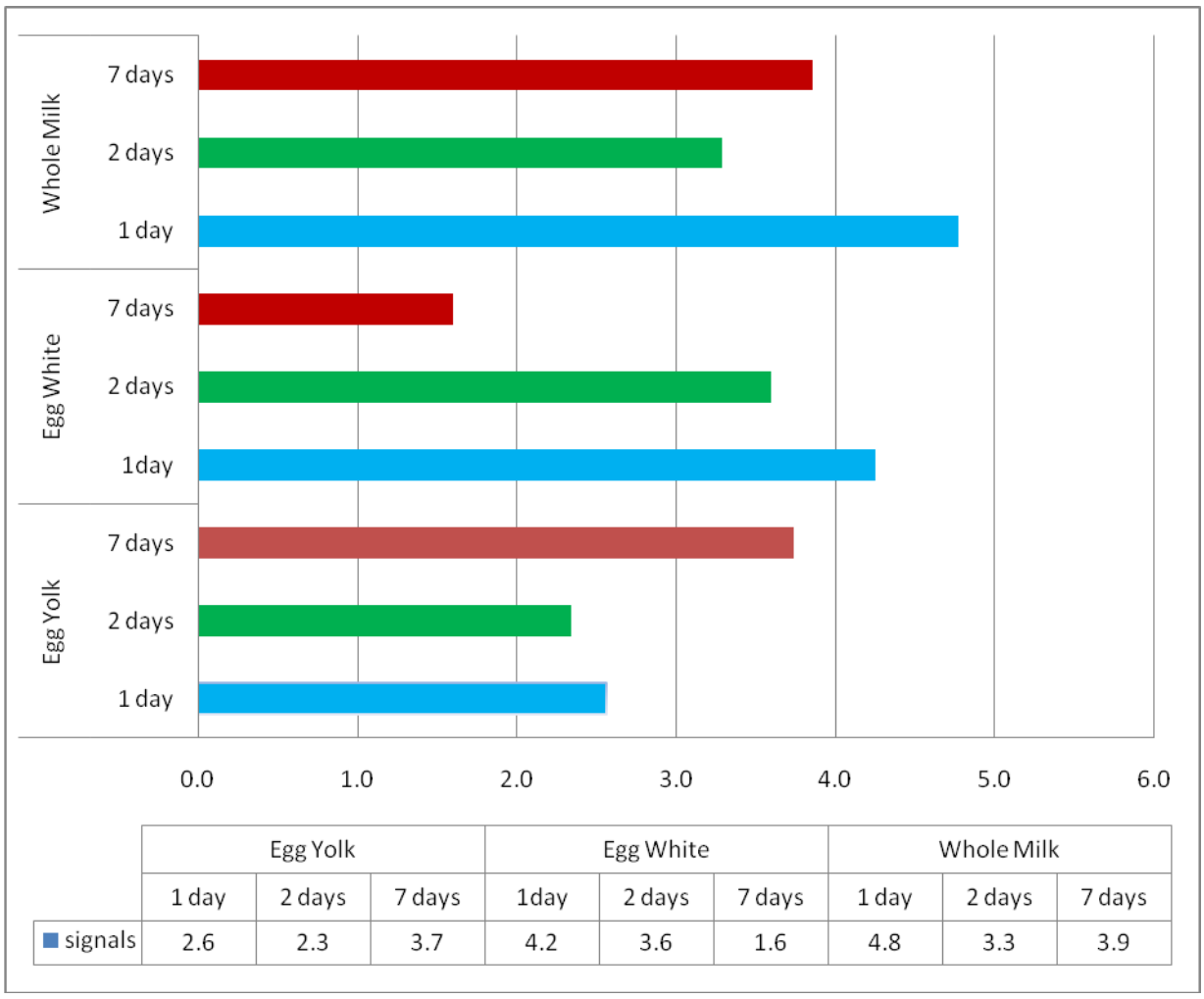
4.4.2 Egg sample:

Results of different storage time for different egg constituents namely; egg white, egg yolk, and whole egg were found to be reasonable (Figure 4.11).

As the full beam of IR wavelength used, the variation among these three compositions were exist with remarkable difference for storage time role. As the storage time increased, three compositions exhibited higher measurements in absorbance. This variation could be for many reasons. The degradation in certain constituents, water loss, and transformation of some materials during storage time all played a role in the measurements values.

However, certain and very specific wavelength must be used enable to specify the component changes.

At the same time, the real samples need to be measured with multi IR-filters. Each filters need to be specific for certain constituents.



	Egg Yolk			Egg White			Whole Milk		
	1 day	2 days	7 days	1day	2 days	7 days	1 day	2 days	7 days
signals	2.6	2.3	3.7	4.2	3.6	1.6	4.8	3.3	3.9

Figure 4.11 Values of Egg with PVDF at period of time.

4.5 Quality Assuring Test:

As this investigation is based on a fact that each food elements of constituents should have one specific wavelength to be measured by. Enable to verify this assumption one measure quality assurance test was conducted to validate this assumption.

As shown in Figure 4.12, measurements of $CaCl_2$ with specific IR-filter (850nm wavelength) showed a remarkable measurements with certain reasonable trend. At the same time when this measurements were carried out with merging the 850nm IR-filter with 720 IR-filter another filter or merging the 850nm IR-filter with 1000nm IR-filter no readings was observed.

This tremendous result revealed the idea and the fact that the food component could have only one specific IR wavelength to be measured with.

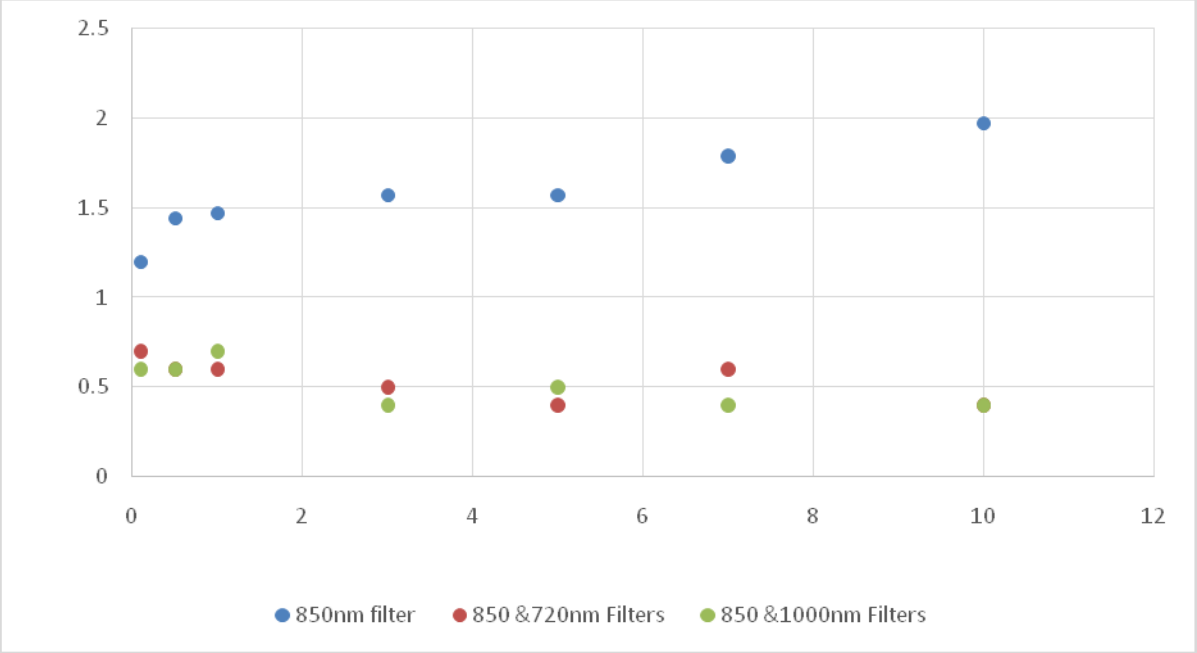


Figure 4.12 $CaCl_2$ sample tested by combined IR-filters in comparison with its specific IR-filter.

5.1 Introduction:

Through the obtained results and several noticeable remarks, several conclusions were drawn from this investigation, further investigation in the future is required to shed more light on the subject.

5.2 Conclusions:

The following conclusions were highlighted from the major findings of this The PVDF film was found to react with organic materials and not limited to certain physical materials.

- IR source was able to create a measurable interaction between food component and PVDF.
- Major food components found to be interacting with specific wavelength.
- The specific wavelength linked with specific food item found to be determined.

5.3 Future Work:

Based on the results obtained in this investigation, some major suggestion for future investigation can be made, as following:

- All specific IR-filters need to be investigated
- Several food items should be involved in one investigation

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ملخص : **استخدام تقنية Photopyroelectric في تحليل الغذاء ومايكروبات الغذاء**

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تم استخدام الفيلم الكهروضوئي المعروف باسم فيلم بولي فينيل دايفلورايد (PVDF) لاختراع تقنية جديدة للكشف عن العناصر الغذائية نوعيا وكميا. العديد من المواد الغذائية وهي: الكربوهيدرات والبروتينات والدهون والمعادن والفيتامينات والمعادن ، وكذلك العديد من الكائنات الحية الدقيقة ، وهي ؛ السالمونيلا ، الإشريكية القولونية ، المكورات العنقودية ، السودوموناس ، الخميرة والعفن.

تم إعداد العديد من تركيزات المواد التي تم فحصها على شكل 0.1 و 0.5 و 1 و 3 و 5 و 7 و 10٪.

وجد PVDF أن تكون فعالة جدا في تحديد التركيزات وكشف وجود العديد من العناصر الغذائية ، كما هو الحال في كلوريد الصوديوم ، CaCl2 ، السكروز ، وتريبتوفان. وقد تم الكشف من خلال الحزمة الكاملة ، في حين تم قياس كل عنصر في جميع التركيزات التي تم فحصها في الطول الموجي المحدد. أظهرت النتائج التي تم الحصول عليها علاقة ملحوظة جدا بين العناصر الغذائية المقاسة. كلوريد الصوديوم ، CaCl2 ، السكروز ، وتريبتوفان والطول الموجي. 720 و 850 و 1000 و 760 نانومتر على التوالي.

وفي الوقت نفسه ، وجد أن قياسات الكائنات الحية الدقيقة ، أي المبيضات ، يمكن قياسها عند 950 نانومتر.

عينات الطعام الحقيقية تم تخزين البيض على شكل بيض كامل ، صفار البيض ، وبياض البيض والحليب في شكل خام وبسترة وتعقيم في درجة حرارة تخزين مختلفة. وأظهرت العينات التي تم اختبارها وجود ترابط كبير بين وقت التخزين وقياسات الامتصاص كمؤشر على نمو أعلى للكائنات الحية الدقيقة عن طريق زيادة وقت التخزين.