

**DEVELOPMENT OF FOOD SENSOR AS AN ANALYTICAL
TOOL IN SELECTED FOODS**

By

MUTHULAKSHMI. R

(16PFD007)

A THESIS SUBMITTED TO THE
AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER
EDUCATION FOR WOMEN

COIMBATORE-641043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

**MASTER OF SCIENCE
IN
FOOD SERVICE MANAGEMENT AND DIETETICS**

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Certified as a Bonafide Research Work


Signature of the Head of the Department
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Signature of the Guide
10/04/2018

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I. INTRODUCTION

The term food sensor is defined as a device including control and processing electronics, to produce an output that is a measure of that quality. Biosensor is a biological detection system consists of a biological component combined with a transducer to perform measurement of a biochemical quantity. Biosensor is an analytical device which integrates the biological elements with the physiochemical transducer to produce an electronic signal is proportional to a single analyte and which is fetched into a detector.

The term biosensor was developed in 1960s to define the use of bio electrodes involving enzyme electrodes that presented and bio catalytic activity. In 1962, Leyland C. Clark brought up the very rudimentary model of biosensors forward with the early invention of an oxygen electrode in which the electrochemical detection of oxygen or hydrogen peroxide could be used for a broad choice of bio analytical purposes. For many different purposes it is used in medico-clinical, environmental, food-agricultural, security and forensic science and other fields are making these devices increasingly popular (Lisdar, 2007).

Biosensors research is booming around the world and the area of applications range from medical to agriculture. The types of instruments required for the agro-food diagnostics market can be divided into large multi-analysers, bench-top portable instruments and one shot disposable sensors. Many of the instrumentations developed to date were for the medical diagnostics market. Fish and meat freshness instruments, based on the determination of nucleotide related compounds to indicate whether the product is fit for human consumption have been introduced to the market.

Biosensors are very useful in food borne pathogen detection. For instance, they have sensitivity in the range of mg/ml for microbial toxins; provide fast or real- time detection; and, the miniaturization of biosensors allow for integration in food production equipment and machinery. The main disadvantage of biosensors is the instability of the biological sensing component, which tends to degrade and lose its effectiveness over a short period of time. This is due to the various stresses encountered within an environment, including pH, temperature or ionic strength (Anand, 2013)

USA remains as the most important country in terms of research in biosensors, followed by China and Germany, the period of last 5 years (2009-2014) China's influence

in this field is gaining momentum and countries like South Korea and India are increasingly researching in these areas with a higher number. This trend will continue with biosensors becoming smaller through miniaturization into the nanoscale range, the number of applications and research devoted to this field will increase (Raveendran, 2015)

The functions of the food packages include protection, containment, communication with the user, ergonomics and marketing. Containment means ensuring the right quantities of products to avoid spills. The communication function is regulated by law and the proper display will influence the consumer acceptance of the product. The information must contain features such as weight, origin, ingredients, nutritional value, precautions for use, mode of transport and recycling or disposal. Trademarks used packaging and labels for promotion, marketing, and product sales Ergonomics in the consumption of a food product is related to minimizing physical effort and discomfort to transport, store, use, and dispose of the container. It has been shown that the physical characteristics and improved containment aspect of food packaging are expectations that affect sales of products and consumer attitudes (Carolina, 2015)

Biosensors in food industry are used for mainly two purposes. First is enzyme biosensor, which is mainly used in liquor and beverages industry for detecting or measurement of carbohydrates from alcohol, amino acids, amines, amides, phenol etc. (Parthasarathy, 2013)

Biosensor technology represents an extremely wide field with a great impact to healthcare, environmental and food quality control. Recently very last years specifically for monitoring food contaminants. Innovation in materials science, nanotechnology and biomimetic design are reinforcing the biosensor field. The current and future trends in materials used for biosensing, miniaturization and development of portable devices in order to have on-site detection of the target analytes(Bala, 2016)

Biosensors have more advantages to detect pathogens in foods rapid and continuous measurement high specificity, very less usage of reagents required for calibration, fast response time and ability to measure non-polar molecules that cannot be estimated by other conventional device, time consuming and we can monitor the pathogen content and spoilage easily.

Food borne illness has become a major threat to public health. Food borne diseases are generally caused by the consumption of food or water contaminated with pathogens which include bacteria, viruses, fungi, toxins, and parasites. There are 31 known pathogens, which cause food borne illness and bacterial pathogens account for the majority of cases resulting in hospitalizations and death. Among food borne bacterial pathogens, *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strains (STEC), and *Vibrio* spp. are the leading causes of food borne illnesses (Feng, 2001)

Milk contains many preservatives and adulterants such as hydrogen peroxide, salicylic acid, benzoic acid, water, neutralizers, melamine and so on. Addition of these two types of substances in the milk. Now for the dairy industry, it seems to be difficult to run the plant without the use of preservatives or adulterants especially under some conditions. In addition to their potential toxic effects on human health (Wilson, 2017).

Artificial sweeteners, high fructose corn syrup, Monosodium Glutamate (MSG /E621), Trans Fat, common food dyes, sodium sulfite (E221),sodium nitrate/sodium nitrite, BHA And BHT (E320) these are the common food additives which mainly effect the health (Saltmarsh, 2000)

Food borne pathogens are detected using through HPLC, ELISA and PCR, it is clear that reduction and/or elimination of cultural enrichment will be essential in the quest for truly real-time detection methods (Breitstein, 2005)

The principle of detection of a biosensor is based on the specific interaction between the analyte and the biological components. As a result of this specific interaction, different properties can be changed and also detected and measured by transducers. The most important characteristics of biosensors are their specificity, high sensitivity, short response time; act as an integrated system.

Antibodies that recognize bacterial pathogens have been used in the direct identification of food borne pathogens or to aid in detection by separating microbial cells from the food matrix, that is, IMS. Bacterial pathogens can be concentrated before culture using IMS. The captured pathogens may then be detected by PCR and other technologies. Because much of the matrix is removed during the IMS, the resulting concentrate has

lower levels of inhibitors and is also of smaller volume, both factors serving to improve the sensitivity of PCR-based tests. Additional platforms of antibody-based assays for detecting pathogens and toxins include latex agglutination, ELISA and biosensor (Meng, 2009)

The development of rapid, competent and reliable methods for direct detection and identification of food borne pathogens. In this overview, we have concentrated specifically on microbe based bio sensing methods such as optical, Surface Plasmon Resonance (SPR), amperometric, potentiometric, whole-cell, electrochemical, impedimetric, piezoelectric for the rapid detection of food borne pathogens (Sindhu, 2011)

Conventional and standard bacterial detection methods such as culture and colony counting methods, immunology based methods and polymerase chain reaction based methods, may take up to several hours or even a few days to yield an answer. Obviously this is inadequate, and recently rapid methods. Although new technologies like biosensors show potential approaches, further research and development is essential before biosensors become a real and reliable choice. New bio-molecular techniques for food pathogen detection are being developed to improve the biosensor characteristics such as sensitivity and selectivity, also which is rapid, reliable, effective and suitable for *in situ* analysis (Adley, 2012).

Rapid detection of food borne pathogens is important for reducing diseases caused by food contaminated with pathogens and toxins. Current methods rely on conventional culture based techniques which are time wise lengthy and require trained personnel and are not effective for on-site use. On the other hand biosensors can detect pathogens in a much shorter time with sensitivity and selectivity comparable to the conventional methods, but with a slightly lower reliability. Since they are low cost and high through put devices and do not require trained personnel, they potentially can be used in future as stand-alone devices for on-site monitoring. Biosensors that use optical, electrochemical and piezoelectric transduction mechanisms for the detection of food borne contaminants (Mutharasan, 2013)

Electrochemical biosensors have shown great promise in the development of rapid methods for the detection of food borne pathogens and have been intensively the advancements made in the development of electrochemical biosensors for the rapid

detection of one of the most common food borne pathogens, *Escherichia coli* O157:H7. The different configurations of electrochemical biosensors based on the sensing principles and measured electrical parameters, as well as the latest improvements of technology in the progress of electrochemical biosensor development to detect *E. coli* O157:H7 (Yanbin, 2016)

Culture methods for detecting bacterial pathogens in food are based on the growth of bacteria on nutrient media. A general procedure includes homogenizing a food sample, enriching pathogens if the concentration of pathogens is low and then separating of the target pathogen on a selective medium was followed by biochemical tests to ensure the presence of the target. The culture methods are sensitive, generally inexpensive and simple. However, they are lab work and may require several days depending on the growth of bacteria (Harris, 2007)

Enzyme biosensors based on the inhibition of cholinesterase enzymes are used to detect traces of organophosphates and carbamides from pesticides that may be present as poisonous and harmful residues on farm produce. Some microbial sensors are selective and sensitive in the detection of ammonia and methane (Bhunja, 2007)

The immunological approaches for the detection of pathogens work on the principle of specific affinity between microbial antigens and monoclonal or polyclonal antibodies. They are used for rapid detection and identification of pathogens, including bacteria, viruses, fungus as well as their toxins. This method is very sensitive, rapid, selective and cost-effective. Latex agglutination and enzyme-linked immuno sorbent assay (ELISA) are the techniques majorly used in food industry for identification of food pathogens like *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, *Escherichia coli* O157: H7, *Listeria* and *Shigella*, *Staphylococcus aureus* (Zhang, 2009)

Microbiological analysis of food is an integrated part of microbial safety management in the food. monitoring and controlling the food borne pathogens are traditionally carried out by conventional microbiological methods based on culture, Polymerase Chain Reaction (PCR) was the analysis allowing detection of pathogenic microorganisms in food, without the necessity of classical isolation and identification. At present, PCR and quantitative polymerase chain reaction (qPCR) were essential analytical tools for research works in the field of food borne pathogens (Cabado, 2015)

The food industry needs suitable analytical methods for process and quality control; that is, methods that are rapid, reliable, specific and cost effective in their provision of information about physical and chemical characteristics of food. Apart from a few important analytes, such as sugars, alcohols, amino acids, flavours and sweeteners, food applications mainly focus on the determination of contaminants. Very few biosensors play a prominent role in food processing or quality control. Considerable effort must be made to develop biosensors that are inexpensive, reliable and robust enough to operate under realistic conditions (Kubota, 2002)

The food industries need rapid and affordable methods to assure the quality of products and process control. Biosensors combining a biological recognition element and a sensitive transducer, are versatile analytical tools that offer advantages as classical analytical methods due to their inherent specificity, selectivity and simplicity. Recent trends in the development and applications of biosensors used in food fermentation industry, focusing on amperometric enzymatic and microbial sensors (Noguer, 2010)

In food quality assessment, antibodies or immunosensors may be used in assays to detect small molecules such as water-soluble vitamins and chemical contaminants. They may also be used to detect any pathogenic organisms present in meat, poultry, eggs and fish (Mandal, 2014)

The inherent specificity, selectivity and adaptability of biosensors make them ideal candidates for use throughout the food industry. Potential applications within the supply chain range from testing of foodstuffs for maximum pesticide residue verification through to the routine analysis of analytes concentrations, such as, glucose, sucrose, alcohol, etc., which may be indicators of food quality/acceptability. Biosensor formats include simple “one-shot” disposable devices that can be used either in the field or integrated into more sophisticated laboratory instruments. Until now, the main impact of these devices has been in the medical diagnostics field. However, with ongoing technical development, the food industry will be one of the prime beneficiaries of biosensor technology in the future (Tigwell, 2005)

Due to specificity, portability, simplicity, high sensitivity, potential ability for real-time and on-site analysis coupled with the speed and low cost, biosensors have been projected to have applications in food analysis, environment control, clinical detection,

drug and agriculture industries etc. Besides this, biosensors offer exciting opportunities for numerous decentralized clinical applications, ranging from emergency room screening, home self testing and alternative site testing, continuous and real-time in vivo monitoring. New generation of biosensors combining new bio receptors with the ever-growing number of transducers is emerging.

The use of biosensor in the food industry may include nutrient analysis, detection of natural toxins and anti nutrients, food process monitoring by measuring enzyme activity and microbial contamination, and rapid detection of genetically modified organisms, proteins, vitamin B complex, essential amino and fatty acids and hazardous residual materials, pesticides and antibiotics (Kubota, 2002)

Biosensors have extensive applications in the food and agriculture industries. The devices contain a transducer and a biological element, which may be an enzyme, antibody, microbe, or organelle. The biological element (bioelement) interacts with the analyte being tested and the biological response is converted into an electrical signal by the transducer. agricultural and food industry(Wang, 2006)

Biological oxygen demand (BOD) analyzers use bacteria such as *Rhodococcus erythropolis* immobilized in collagen or poly acrylamide. These devices are widely used to test the quality of waste water. BOD biosensors can analyze 2 to 20 samples every hour (D'Souza, 2001)

Biosensors may be used to measure carbohydrates, alcohols and acids in fermented foods. The devices are mainly used for quality control processes in food production. The devices, however, need to be kept sterile, frequently calibrated and require analyte dilution. Enzyme based biosensors can be used in food quality control to measure amino acids, amides, amines, carbohydrates, heterocyclic compounds, carboxylic acids, gases, inorganic ions, cofactors, alcohols and phenols. Biosensors can also be used in the assessment and analysis of produce such as wine, beer and yoghurt (Reilly, 2005)

Biosensors are an important alternative in the food industry to ensure the quality and safety of the food products and process controls with effective, fast and economical methods. The use of enzymatic biosensor technology in the food processing, quality control and on-line processes is compared to conventional analytical technique (Wilkins, 1999)

Recent study was carried out using the Resonant Circuit Sensors for monitoring of bacteria growth to control the food quality by using printed thin or thick film sensor comprised of an Inductor Capacitor (IC) resonant circuit the sensor is placed within the biological medium and remotely detected through using loop antenna, measures the complex permittivity of the medium and bacteria growth increases the complex permittivity of a biological medium the LC sensor. By using this sensor they found the different bacterial strains of *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas putida* in milk, beer and meat (Ashok, 2005)

Rapid advances in biosensors have recently been reported. This has been possible due to rapid growth in the development of new biomaterials such as conducting polymers, copolymers and sol gels etc and the reported improvements in sensing techniques. Biosensors are miniaturized devices employing biochemical molecular recognition as the basis for a selective analysis. The response generated as a result of biochemical reaction is detected by a transducer to give a signal (optical/electrical/thermal) that can be used with or without amplification for the estimation of the concentration of the given sample. Among the various biosensors electrochemical sensors, especially amperometric biosensors presently hold a leading position (Salazar, 2015)

Biosensors are classified according to the transduction mechanisms and biological receptor mechanism. The biosensor may be divided into basic four main groups based on the transduction mechanisms which include optical, mass, electrochemical and thermal biosensor. In addition, the biological component are used in biosensors include enzymes, microbial, immune and nucleic acids, biochips and bio mimetic sensors, can utilize artificial bio recognition elements (Merotra, 2016)

The type of biosensor including bio elements such as an enzyme, antibody or a cell receptor and a sensing element or a transducer. These two elements are combined together through a number of methods such as covalent bonding, matrix entrapment, physical adsorption and membrane entrapment (Grimes, 2000)

Alcohol sensor is used for detecting alcohol concentration, it is highly sensitive and it gives response fast. The sensor provides an analog resistive based on alcohol concentration. The drive circuit of alcohol sensor is very simple and it needs all in one resistor. Sensitive materials of MQ-3 gas sensor have less conductivity in clean air but it

has high sensitivity to alcohol gas and it can be resistant with the interference of gasoline, smoke and vapour(Fraden, 2016)

Gas sensor continuously react to the gas present and it may get alters according to the molecular gaseous state. The gas module consist of steel exoskeleton under the sensing element was housed. The sensing element was subject to current through connecting leads current is known as heating current. The gases comes sensor to the sensing element gets ionized and absorbed by the sensing element. It alters the value of the going out and changes the resistance of the sensing element. The connecting leads of the sensor are thick and t can be connected firmly to the circuit through that sufficient amount of heat gets conducted to the inside parts. They are casted from copper and have tin plating over them. This unit will be incorporated easily into a alarm zone. It gives the signals through sound or displaying the visual indication of a gas concentration. The sensor have high sensitive and its response quickly (Sberveglieri, 1992)

Temperature sensor will senses the various levels of temperatures. It is used for the experimental studies and purposes. It gives the reading in centigrade or degree Celsius. A digital temperature sensor provides 9 – bit temperature reading. It gives a accurate reading. The sensor is designed to read the value from 0° to 70° C. It is possible to read $\pm 0.5^{\circ}$ C. occasionally. The digital temperature sensor consist of three terminals and it requires maximum 5.5 V supply. This type of sensors will perform the operation to alter its resistance according to the various temperatures. It calculates temperature through the change in resistance which is sensed by circuit. If the voltage increases and there is also increase in temperature. The temperature sensor is directly connected to microprocessor input and it's capable of direct and reliable communication with microprocessor (Wilson, 2005)

Present study focused to develop the food bio sensor to detect the microorganism that is commonly present in foods. This work involves in the detection of spoilage seen in certain foods and water due to contamination of microbial bacteria which can be detected easily from the presence of acids and gases present in the head space of the spoiled foods and contaminated water which is not present in fresh foods and water before it gets spoiled.

The present study focuses with the following objectives

- Develop the food sensor using Gas and Temperature sensor
- Detect food additives and micro organism in the selected foods.
- Avoid health risks among peoples.

II. REVIEW OF LITERATURE

The review of literature pertaining to the present study “**Development of Food Sensor as an Analytical Tool in Selected Foods**” under the following headings

A. Biosensors as an Analytical Tool

B. Different Components Present in Biosensors.

C. Different Methods Used in Detecting Micro organisms, Food additives, Adulterants in Common Foods.

D. Role and Benefits of Biosensors in Food Industry

A. Biosensors as an Analytical Tool

The importance of analyses of different parameters in food products and monitoring of a production process requires quick and reliable analytical methods and devices. For this purpose, biosensors can be a suitable option, whereas most of the current quality control techniques are time consuming, expensive and unpractical. Biosensors developed for analysis of different components present in food samples, namely glucose, fructose, sucrose, lactose, lactic acid, malic acid, acetic acid, ascorbic acid, citric acid and amino acids, ethanol, glycerol, and triglyceride. Biosensors showed desirable sensitivity, selectivity, and response time required for various applications. They are often designed to avoid interference from components present in a complex sample to be analyzed (Sturdik, 2011)

A biosensor comprises two distinct elements: a biological recognition element (e.g. antibodies, enzymes, lectins, receptors and microbial cells) and in close contact, a signal transduction element (e.g. optical amperometric, acoustic and electrochemical) connected to a data acquisition and processing system. Thus the signal from the biological element is converted to a quantifiable signal, e.g. electrical. According to Thevenot et al (1999), an electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element. Because of its ability to be

repeatedly calibrated, it should be distinguished from a bio-analytical system, which requires additional processing steps, such as reagent addition. The authors further suggest that biosensors may be classified according to the biological specificity conferring mechanism or alternatively, to the mode of physic-chemical signal transduction and that standard protocol for setting performance criteria of biosensors are also important (Patel, 2002)

A biosensor is an analytical device that integrates a biologically derived recognition of molecules such as enzymes, antibodies, phages, aptamers, or single-stranded DNA with a suitable physicochemical transducer. Electrochemical, optical, thermometric, piezoelectric, and magnetic transducers are the common types. Apart from these biosensors, there are immuno chromatographic devices, which do not use any of the transducers mentioned above.

These devices are based on lateral flow strips and they are ultra sensitive tests and enable for on-site visual detection of target molecules. In this field, the most successful lateral flow strip products are the home pregnancy strip and glucose testing strip, which have been widely commercialized. Biosensors generally produce an electronic or optical signal proportional to the specific interaction between the target and the recognition molecule immobilized on the biosensor. They also transform biological interactions into electronic signals that can be properly measured and recorded. A wide range of subjects from small protein molecules to large pathogens can be detected by biosensors. In biosensor types, electrochemical biosensors are more suitable for onsite analysis and also they can easily be miniaturized for handheld devices. A commercial biosensor, except for immuno chromatographic devices, generally consists of a detector and a sample application and delivery system. All of them vary depending on the manufacturer and the related application (Bahadır, 2015).

A biosensor is an analytical device which incorporates a biological sensing element integrated within a physicochemical transducer. The aim of a biosensor is to produce an electronic signal proportional to the specific interaction of analytcs with the sensing element. This means that biosensors essentially transform bio-molecular interactions into a digital signal and thereby can be used for detection of analytcs ranging from small molecules to whole of pathogenic micro organisms (Skottrup, 2008)

The amplifier in the biosensor responds to the small input signal from the transducer and delivers a large output signal that contains the essential waveform features of an input signal. The amplified signal is then processed by the signal processor where it can later be stored, displayed and analysed. Biosensor has been proved to be rapid, economic consistent assessment technique, which has expanded in many food industries (Borty, 2015)

Biosensors act as analytical devices employing a biological material or biomimic as a recognition molecules integrated within a physicochemical transducer or transuding micro-systems. The outcome of this is a digital electronic signal proportional to the concentration of a specific analytic or analytics. Biosensors are used in various fields, with miniaturization and reduced cost has further increased the analytical capabilities of such device.

B. Different Components Used in Biosensors.

Electrochemical biosensors is a simple device. It measures the measurement of electronic current, ionic or by conductance changes carried by bio-electrodes (Conier, 2015)

The Amperometric biosensors are based on the electrons movement, i.e. electronic current determination as a reaction of enzyme-catalyzed redox reaction. Generally a normal contact voltage passes through the electrodes to analyze. In the enzymatic reaction which produces the substrate or product can transfer the electrons with the surface of electrodes to be reduced. As a result an alternate current flow can be measured. The substrate concentration is directly proportional to the magnitude of the current. The reduction of oxygen is acquired through the oxygen electrodes and it is a simple way to from an amperometric biosensor. The example is the determination of glucose by glucose. The above description is about the first generation of amperometric biosensor and it has a direct transfer of electrons which are released from the electrodes are having some difficulties. The second generation amperometric biosensors are developed in a mediator takes the electrons and transfer to the electrodes (Yao, 2008)

The blood glucose biosensors are used widely throughout the world for diabetic patients. It has a single use disposable electrodes with glucose oxide and derivatives of a mediator (Ferrocence) and the shape of the blood glucose biosensor looks like a watch

pen. With the help of hydrophilic mesh electrodes are converted. The Blood glucose Biosensor is a good example of amperometric biosensor (Ngo,2000)

In potentiometric biosensors changes the concentration of ionic is determined by the ion-selective electrodes in this pH electrodes are used most commonly. Hence a large amount of enzymatic reactions is involved in the release of hydrogen ions. Ammonia-selective and carbon dioxide selective electrodes are some other important electrodes. The potentiometric electrode and the reference electrode can be measured with the help of potential difference and it is directly proportional to the substrate concentration. The Potentiometric Biosensors is the sensitivity of enzymes to ionic concentration like H^+ and NH_4^+ The ion- selective field effect transistors are lower price devices. It can be used in the miniaturization of Potentiometric Biosensors. The example of the ISFET Biosensor is to monitor intra-myocardial for open heart surgery (Yang, 2000)

In conductometric biosensor the biological system there are several reactions that change the ionic species. The electronic conductivity can be measured with the help of an ionic species. The example of the conductometric biosensor is the urea biosensor which utilizing the immobilized areas. The following reactions show the urea catalyses. The given reaction is associated with the drastic alteration in ionic concentration and they are used for the monitoring urea concentration. In generally during the dialysis and renal surgery the urea biosensor is very useful.

There are many more biological reactions are connected with the production of heat and it forms the basis of thermometric biosensors. The representation of a thermal Biosensor and it consists of a heat insulated box fixed with heat exchange.

The optical Biosensor is a device, it utilizes the principle of optical measurements like fluorescence, absorbance and etc. They used in fiber optics and optoelectronic transducers. The optical biosensors are safe for non electrical remote sensing of materials. In the transducer elements primarily optical biosensors involves in the enzymes and antibodies. Usually the Biosensors is not required any reference sensors and the comparative signals are generated by using the sampling sensor. The important Biosensors is described briefly (Joshi, 2006)

The working of the fiber optic lactate biosensor is based on the measurement of change in oxygen concentration, molecular by identifying the effects of oxygen in fluorescent dye. The following reaction is reduced by the enzyme lactate mono-oxygenase.

The oxygen depends on the amount of fluorescence generated by the dyed film this is because of oxygen has a reducing effect on the fluorescence. In the reaction mixture the concentration of lactate is increased, oxygen is utilized and as a result, there is a proportional decrease in the quenching effect. Hence there is an increase in the fluorescence output can be measured

For the diabetes patients the blood glucose is more important to monitor. In this simple technique is used, i.e. Paper strips saturated with the reagents it contains glucose oxide, Horseradish Peroxidase and a Chromogen. The following reactions take place. Using the portable reflectance meter it can measure the intensity of the colour of the dye. In the world wide the glucose strip industry is very high. The calorimetric test strips of cellulose covered with the suitable enzymes and reagents are in use for the view of more blood and the urine parameters. The other optical fiber Biosensors are used in the devices of optical Bio sensing it measures the p CO₂ and in critical care and in surgical monitoring(Rogers, 1996)

The principle of piezoelectric biosensor is used in sound vibrations, hence it is called acoustic Biosensors. The basics of the biosensors are formed by the piezoelectric crystals and the characteristic frequencies are trembling with the crystals of positive and negative charge. By using the electronic devices we can measure the certain molecules on the crystal surface and alters the response frequencies using these crystals we can attach the inhibitors. The Biosensors for cocaine in the gas phase has been developed by attaching the antibodies cocaine to the surface of the crystal.

The immune biosensors works on the principle of immunological specificity and mostly coupled with measurement on the potentiometric biosensors. There are different configuration of probabilities for immune Biosensors some of them are given below and the figure shows the description. The immobilized antibody can directly combine through the antigen. The immobilized antigen can combine with the antibody which can twist to a second free antigen. The immobilized antibody combined with the free antigens and enzyme labeled antigen in opposition (Ksha, 2013)

Even many centuries ago, people already had a great desire to know the temperature. Reference points could be found, such as freezing point of water or the body temperature. However, to determine between those fixed points a temperature meter or

thermometer was needed. This led to the invention of the mercury- in – glass thermometer, which is still widely used today. Another issue was the definition of the scale. In 1714, Fahrenheit proposed a scale that takes the minimal possible temperature he could imagine to appear in central Europe as 0°F (- 18°C) and the body temperature as 96°F. Celsius proposed a few years later in 1742 a similar scale but he took the freezing point of water as 0°C and its boiling point as 100°C. Both scales are still in use, but the Celsius scale is the most widely used scale. More than a Century later, in 1851, Kelvin proposed his law of thermodynamics in which he proved that the theoretically minimal possible temperature is – 273. 15°C. Since that time, scientists have often used the Kelvin scale, which is a Celsius scale that is shifted (Huijsing, 2013)

C. Different Methods Used in Detecting Micro Organisms, Food Additives and Adulterants in Common Foods

The food industry needs suitable analytical methods for process and quality control; that is, methods that are rapid, reliable, specific and cost-effective in their provision of information about physical and chemical characteristics of food. Apart from a few important analytes, such as sugars, alcohols, amino acids, flavors' and sweeteners, food applications mainly focus on the determination of contaminants. However, very few biosensors play a prominent role in food processing or quality control. Considerable effort must be made to develop biosensors that are inexpensive, reliable and robust enough to operate under realistic conditions (Bouvrette,1997)

Food borne pathogens are a growing concern for human illness and death. There is increasing demands to ensure safe food supply. There is continuous development of methods for the rapid and reliable detection of food borne pathogens. Advent of biotechnology has greatly altered food testing methods. The possibilities of combining different rapid methods, including immunological and DNA based methods should be further exploited.

Further developments in immunoassays and PCR protocols should result in quantitative detection of microorganisms and the simultaneous detection of more than one pathogen or toxin. Lastly technology continuing to advance at a great pace, the next generation of assays currently being developed potentially has the capability for near real time and online monitoring of multiple pathogens. Modern methods are based on molecular biology techniques like PCR, RFLP, DNA micro assay, immunological

techniques like ELISA, biophysical and biochemical principles with the application of bio sensors like bioluminescence sensor, bio-analytical sensors utilizing enzymes, electrical impedometry and flow cytometry conventional methods and newly developed rapid pathogen detection techniques and rapid methods (Pal, 2011)

Detection of food borne pathogens is considered to be the “gold standard,” they remain cumbersome and time consuming. Despite the advent of rapid detection methods such as ELISA and PCR, it is clear that reduction and/or elimination of cultural enrichment will be essential in the quest for truly real-time detection methods. As such, there is an important role for bacterial concentration and purification from the sample matrix as a step preceding detection, so-called pre-analytical sample processing (Dwivedi, 2010)

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 labour by automating methods whenever possible (Jantzen et al. 2006)

In spite of its importance the microbiological analysis of food has many limitations. Uncertainty of the analytical result must be considered when establishing microbiological criteria, including the variance associated with the sampling plan, method of analysis, and laboratory performance (Betts and Blackburn,2009) .The microbiological analysis of food remains a challenging task for virtually all assays and technologies, especially for particular pathogenic species (Feng 2007)

The problems may be due to the complexity of food matrices and composition, the heterogeneous distribution of low levels of pathogens, the stress suffered by the microorganisms during the processing of foods, the presence of bacteria from the normal

micro-biota, especially in raw foods. The complexity of food matrices remains the major obstacle to the development of effective sampling and rapid testing methods (Feng 2007).

Long duration enrichments are often used due to the low number of pathogenic microorganisms that tend to be present in food samples. Although previous enrichment is a limitation in terms of assay speed and precludes quantification of the original contaminant, it provides separation and concentration of microorganisms present in Food 15 essential benefits, such as diluting the effects of inhibitors, allowing the differentiation of viable from nonviable cells, and allowing for the repair of cell stress or injury that may have resulted during food processing (Jantzen et al. 2006) Hence, it would be difficult to completely eliminate enrichment culture from the process of pathogen detection in foods (Alonso 2012)

Bacteria are a major source of contamination in water and food supplies resulting in both food poisoning and disease outbreaks Although constant monitoring of bacteria concentrations can reduce food-related illnesses, counting bacteria in a biological food medium is a time consuming and *Sensors* difficult task due to the complex nature of bacteria and the biological medium. Today most bacteria counts are still determined by standard plate count (SPC) or psychotropic bacteria count (PBC) which are labour and time intensive. Microbiological impedance devices provide a less labour intensive way to estimate bacteria count in a biological medium. Microbiological impedance devices measure the permittivity and/or impedance spectra of the biological medium by immersing two or three electrodes into the medium and measuring the voltage at a constant current. The bacteria concentration or even the strain of the bacteria can then be calculated from the measured permittivity and/or impedance spectra (Pukket, 2002)

One of the major applications of microbiological impedance devices is to determine the initial bacteria count in a food medium through a technique called Impedance Detection Time (IDT). The initial bacteria count obtained from IDT is then used to estimate the useful shelf life of a food product. However, the initial bacteria count does not always correlate well with the real shelf life due to improper storage conditions or post packaging food contamination. Furthermore, direct characterization of bacteria concentrations using impedance devices has some disadvantages such as penetration of the test chamber which introduces the potential for contamination, polarization of the probe electrode and bubble formation at the electrode surface. An optimal way to monitor food

quality is to remotely measure the bacteria population just prior to food consumption (Bachas, 2002)

Biosensors can be directly applied for the detection of pathogen in processed food matrices. Such processing methods usually include mincing and homogenization of food samples in the presence of detergents and/or photolytic enzymes and the choice of processing method depend on the type and complexity of the sample. Biosensors do not require the time consuming sample pre enrichment and secondary enrichment steps and therefore can accurately predict the level and kind of food contamination much faster compared to conventional microbiological, immunological and molecular biological methods (Evoy, 2013)

Higuchi et al., (1993) constructed a system that detects PCR products as soon as they accumulate. This was the beginning of the real time or qPCR, also known as the second generation of PCR. qPCR allows the monitoring of the amplification process in real time by using fluorescence, and not only at the end of the reaction as occurs in conventional PCR. Its major advantages are that it is a fast and easy-to-perform analysis, a single reaction by using different sets of primers.

This option saves considerable time and effort and decreases the number of reactions that need to be performed to detect the desired targets in the sample. However, the presence of many PCR primers in a single tube can cause some problems, such as the increased formation of misprimed PCR products, known as “primer dimers,” and the amplification of unspecific DNA fragments. Primers should be chosen with similar annealing temperatures for multiplex PCR assays. Also, the lengths of amplified products should be similar, since large differences in the lengths of the target DNAs may favour the amplification of shorter targets over the longer ones, which results in different yields of amplified products. In standard PCR protocols, amplification products are detected by gel electrophoresis using UV luminescence and the ethidium bromide double-stranded DNA intercalating dye or similar non-toxic compounds. These procedures have the big disadvantage of relying on end point analysis; therefore, amplification products cannot give quantitative information regarding the initial amount of target molecules (Elnifro et.al, 2000).

Digital PCR (dPCR) allows the precise quantification of nucleic acids, facilitating the measurement of small percentage differences and quantification of rare variants. It was reported to be more reproducible and less susceptible to inhibition than qPCR. Its main

applications are: rare variant measurement, molecular counting, and applications that need higher precision. Due to its growing interest, a “Minimum Information for Publication of Quantitative dPCR (Huggett , 2013)

Conventional methods for the detection and identification of microbial pathogenic agents mainly rely on specific microbiological and biochemical identification by the culture and colony counting methods involve counting of bacteria, immunology-based methods involve antigen–antibody interactions and the third polymerase chain reaction (PCR) method which involves DNA analysis. While these methods can be sensitive, inexpensive and give both qualitative and quantitative information of the tested microorganisms, they are greatly restricted by assay time; also initial enrichment is needed in order to detect pathogens which typically occur in low numbers in food.

Immunological detection with antibodies is perhaps the only technology that has been successfully employed for the detection of bacterial cells, spores, viruses and toxins alike Methods based on antigen antibody bindings are widely used for determining food borne pathogens. Immunology based methods has been used for the detection of *Escherichia coli* (Adley, 2010)

The culturing and plating method is the oldest bacterial detection technique and remains the standard detection and quantification method. Other techniques are necessary because culturing methods are excessively time consuming. For bacterial detection, four to nine days are needed to obtain a negative result and between 14 and 16 days for confirmation of a positive result (Brooks *et al.*, 2004). This is an obvious inconvenience in many industrial applications, particularly in the food sector. Different selective media are used to detect particular bacterial species. They contain inhibitors (in order to stop or delay the growth of non-targeted strains) or particular substrates that only the targeted bacteria can degrade or that confers particular colour to the growing colonies (Borty, 2015)

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Biosensors do not require the time consuming sample pre enrichment and secondary enrichment steps and therefore can accurately predict the level and kind of food contamination much faster compared to conventional microbiological, immunological and

molecular biological methods. A typical biosensor has three associated components: the sensor platform functionalized with a bio-probe to impart specificity of recognition, a transduction platform that generates a measurable signal in the event of analytic capture and the amplifier which amplifies and process the signal to give a quantitative estimate of analytic capture. Biosensors for monitoring food and water samples have not yet been commercialized, unlike those available for medical diagnostics, yet the recent developments show tremendous possibility. This review will discuss different bio-probes and transduction platforms that have been successfully leveraged for pathogen detection with a focus on recent advances in biosensor technology for on-site detection (Poshtiban, 2013)

An ideal biosensor should detect target molecules directly without the use of labelled ligands or multiple washing steps. There are several bottlenecks to be solved in order to perform an efficient analysis. The first is the use of proper surfaces. The second is to efficiently and uniformly print the capture probe (antibody, binding proteins, aptamers) and to have a constant probe density in order to obtain the detection of targets with high reproducibility. The third is to obtain a high sensibility even at a low concentration of the targets. A number of methods are used for the rapid detection of biomolecules in solution. These include immunoassays, chromatographic methods, magnetic and biological biosensor methods (using screen-printed biosensors with immobilized enzymes), DNA biosensors and antibody based detection methods (Maruccio, 2014)

A new DNA chip, based on the use of a ligation detection reaction coupled to a universal array, developed to detect and analyze, directly from milk samples, microbial pathogens known to cause bovine, ovine and caprine mastitis or to be responsible for food borne intoxication or infection, or both. Probes were designed for the identification of 15 different bacterial groups: *Staphylococcus aureus*, *Streptococcus agalactiae*, nonaureus staphylococci, *Streptococcus bovis*, *Streptococcus equi*, *Streptococcus canis*, *Streptococcus dysgalactiae*, *Streptococcus parauberis*, *Streptococcus uberis*, *Streptococcus pyogenes*, *Mycoplasma* spp., *Salmonella* spp., *Bacillus* spp., *Campylobacter* spp., and *Escherichia coli* and related species. These groups were identified based on the 16S rRNA gene. For microarray validation, 22 strains from the American Type Culture Collection or other culture collections and 50 milk samples were tested. The results demonstrated high specificity, with sensitivity as low as 6 fmol. Moreover, the ligation detection reaction universal array assay allowed for the identification of *Mycoplasma* spp.

in a few hours, avoiding the long incubation times of traditional microbiological identification methods. The universal array described here is a versatile tool able to identify milk pathogens efficiently and rapidly (Moroni, 2009)

The computational simulation was used to determine the bio mimetic peptide based sensing material to be deposited on the quartz crystal microbalance (QCM) sensor. Tripos/Sybyl[®]8.0 was used to predict the binding site of an olfactory receptor and determine the binding affinity as well as orientation of the selected ligands (specific molecules) to the olfactory receptor. The designed polypeptide sequence based on the simulation program was synthesized and used as a sensing layer in the QCM crystal. The developed QCM sensors were sensitive to 1-hexanol as well as 1-pentanol as predicted by the simulation algorithm. The estimated lower detection limits of the QCM sensors for detecting 1-hexanol and 1-pentanol were 2–3 ppm and 3–5 ppm, respectively. This study demonstrates the applicability of simulation based peptide sequence that mimics the olfactory receptor for sensing specific gases (Malik, 2011)

The prototype Milk-sense: a volatile sensing system recognizes spoilage bacteria and yeasts in milk. An electronic nose unit including 14 conducting polymer sensors was used to detect the volatile profiles produced by inoculated skimmed milk media or that inoculated with bacteria (*Pseudomonas aureofaciens*, *P. fluorescens*, *Bacillus cereus*) or yeasts (*Candida pseudotropicalis*, *Kluyveromyces lactic*) when grown for 5 h at 30°C. Using discriminant function analyses it was possible to separate unspoiled milk and that containing spoilage bacteria or yeasts. The sensor array used was a useful discriminator of microbial volatile profiles. Quantitative differentiation between three different concentrations of *P. aureofaciens* (10^6 , 3.5×10^8 , 8×10^8 CFUs ml⁻¹) was also investigated and showed that the system could effectively differentiate between treatments. Using an initial inoculum of about 10^3 – 10^4 CFUs ml⁻¹ it was possible to discriminate between unspoiled milk, yeasts and bacterial species (*S. aureus*, *B. cereus* and the *Pseudomonas spp.*) using principal component analyses, and also between the bacteria, the unspoiled milk, and the two yeasts *C. pseudotropicalis* and *K. lactic* with 85% of the data accounted for. The potential for differentiation between four of the five individual bacterial and yeast species was analysis after 5 hour growth at 25°C by using a three-layer back propagation neural network of 46 input sensor parameters. This showed that it was possible to recognize, and differentiate, between species, the butane and milk medium controls. (Chrysanthakis *et al*, 2010)

Evaluation of the distribution capability of food-borne pathogens existing in food products by taking the advantage of quantum dots (QDs) for their photoluminescence properties was carried out. Bacteria namely *Escherichia coli* (*E. coli*) labelled with CdSe-QDs were examined both on an Agar nutrient and ground fish substrates in order to observe their growth rate in different environments. A sample with an appropriate concentration ratio 10^7 CFU/mL of bacteria/CdSe-QDs was empirically selected from the samples which were grown on the Agar containing plates. The selected sample was also tested on a ground fish substrate as a real food sample. The bacterial growth was observed under the irradiation of UV light and the growth patterns were investigated for 3 successive days. The growth patterns indicated that *E. coli* can stay alive and can be distributed on food products so that the growth can be easily monitored. This approach makes bacterial growth on food products detectable so that it can be used as a bacteria-QD assay for an easy detection of food borne pathogens grown on a food sample (Kezami, 2010)

The system consists of various sensors and RF wireless units. The basic units is a sensory system which is directly exposed to the milk samples from 3cm distance where the responses of each sensors are observed from the milk surface. The sensors collects data samples from the spoiled raw milk and this data is processed in the form of gas molecules. If the input data of the gas molecules is in the form of (parts per million) to voltage then by using the heating elements the above data is considered for the further process. The heating element is heating as per the concentration of the molecules and it depends on the molecules levels to convert vapour gas in the form of voltage (Lingam, 2010)

The voltage values are varying continuously according to the concentration of the spoiled milk where vapour gas molecules are represented with parts per million. The sensors are connected to the LPC 2148 IC which presents the processing of the data values in the form of voltages. The concentration of gases and temperature in raw milk is determined using sensors and displayed in the LCD. The total data can be monitored in the PC by using the RF ZigBee module where it is used to transfer the data from ARM 7 unit to central server by using the peer to peer protocol. (Rayappan et al, 2010)

A food additive is defined as any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such

food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods. Many food additives are naturally occurring and some are even essential nutrients; it is the technical purpose that leads to these being classified as food additives and given an E number

Additives carry out a variety of useful functions which we often take for granted. Foods are subjected to many environmental conditions, such as temperature changes, oxidation and exposure to microbes, which can change their original composition. Food additives play a key role in maintaining the food qualities and characteristics that consumers demand, keeping food safe, wholesome and appealing from farm to fork. Food additives are very carefully regulated and the general criteria for their use is that they perform a useful purpose, are safe and do not mislead the consumer (McCann et al. 2007)

Food adulteration is a global concern and developing countries are at higher risk associated with it due to lack of monitoring and policies. However, this is one of the most common phenomena that had been overlooked in many countries. Unfortunately, in contrast to common belief, milk adulterants can pose serious health hazards leading to fatal diseases. This paper presents a detailed review of common milk adulterants as well as different methods to detect the adulterants both qualitatively and quantitatively. This study is organized to be an 'adulterant based' study instead of 'techniques based' one, where qualitative detection for most of the common adulterants are enlisted and quantitative detection methods are limited to few major adulterants of milk. Apart from regular techniques, recent development in these detection techniques has also been reported. Nowadays milk is being adulterated in more sophisticated ways that demands for cutting edge research for the detection of the adulterants. (Ahmed, 2016)

Liquid milk has been an important human nutrient source for a thousand years. However, the safety of milk has always being challenged due to illegal use of preservatives and adulterants such as hydrogen peroxide, salicylic acid, benzoic acid, water, neutralizers, melamine and so on. Addition of these two types of substances in milk has become a common feature for full filling demands of over populated countries. Now, for the dairy industry, it seems to be difficult to run the plant without the use of preservatives and or adulterants especially under Indian conditions. In addition to their potential toxic effects on human health, they also undermine the economy of a country. The lack of timely action against the unscrupulous trades by the public health.

Departments and other law enforcement groups and lack of simple and rapid methods for detection of adulteration further encourage this menace. This overview attempt to address the key features of these two types of harmful substances in milk, their effect on milk composition and constituents along with their regulatory and safety aspects (Singh, 2015)

Milk adulteration, poor hygiene, malpractices, lack of preservation technology, cooling facilities and sanitation conditions are the main causes of losses in quantity and adulteration of milk is the most pressing public health issue. Adulteration of milk is done to increase its volume and then starch and other reconstituted milk powders are added to increase its viscosity. To increase the shelf life of milk dirty ice and some chemicals like hydrogen peroxide, carbonates, bicarbonates, antibiotics, caustic soda and even the most lethal chemical formalin is also being used (Mansour 2013)

Unsanitary handling of foods has been commonly found to be the source of contamination. The carriers of pathogens like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* who eventually transfer these food borne hazards to the consumers. The hands of the food handlers are the most important vehicle for the transfer of organisms from faeces, nose, and skin to the food. The finding that *Salmonella*, non-typhi salmonellae, *Campylobacter* and *E. coli* can survive on finger tips and other surfaces for varying periods of time and in some cases even after washing, supports the reports of contamination of street vended food with toxigenic *S. aureus*, the major being supportive lesions of human beings and the environment.

The lack of facilities for liquid drainage, wastewater and garbage disposal encourages wastes to be thrown into nearby streets and gutters. Such areas act as habitats for rodents, breeding points for flies and media for growth of microorganisms and can transmit to the foods (Rane, 2011)

A leading cause of contamination worldwide is microbial toxins along with agrochemicals. Any pathogenic microorganism in food can lead to severe health consequences in animals and humans. Most prominent food borne pathogen includes mycotoxins, exotoxins and enterotoxins from *Escherichia coli* O157:H7, some strains of *Staphylococcus aureus*, *Shigella* spp., *Bacillus anthracis* (produces anthrax toxin), *Campylobacter jejuni*, *Clostridium perfringens*, *Clostridium botulinum* (produces a powerful paralytic toxin botulin), *Salmonella* spp., *Listeria monocytogenes*, *Vibrio cholera*, *Yersinia enterocolitica* and *Coxiella burnetii* (Chaudhury, 2011)

Milk borne pathogens and their associated illnesses, and several other important microorganisms in milk. A table summarizing the disease characteristics of the major milk pathogens is presented at the beginning of this section, followed by a more detailed discussion of the microorganisms in milk, listed in alphabetical order: *Brucella* spp., *Campylobacter jejuni*, Coliforms, *Coxiella burnetii*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Mycobacterium bovis* and tuberculosis, *Mycobacterium paratuberculosis*, Psychrotrophic Bacteria, *Salmonella* spp., and *Yersinia enterocolitica*. Cases of human illness associated with the consumption of dairy products are listed in the Disease Outbreaks Associated with Milk Products Section. (Boor. 1997)

D. Role & Benefits of Biosensors in Food Industry

Food is a basic requirement of all human beings, microorganisms such as pathogens, virus, and bacterium present in foods and partially or completely destroys the nutritional quality of the foods. Food borne pathogens are a major threat to food safety pathogens can cause serious diseases that leads to the 40 percent of the total 50 million deaths annually in the world, food production, preservation and prevention from chemical and microbial contamination, insect infection and pathogenic contamination have very essential in today's fast life style for food safety and quality, various conventional analytical techniques are used such as polymerase reaction, culture and colony based method and immunology – based methods. These methods are more time consuming and requires a well trained persons,. There is an increasing demand and developing a fast and sensitive technique to monitor food borne pathogens (Neelam, 2015)

Food processing industry faces various challenges; one of the foremost challenges is the need for quick and cost effective methods to detect the presence of allergenic components and pathogens in the food. Biosensors pave way for the rapid detection of pathogens, allergens as well as pesticide residues in food. Detection of contaminants, verification of product contents, product freshness and monitoring of raw materials conversion are the areas of potential biosensor applications. Generally in the food and the agricultural industries, chemical and microbiological analyses are done periodically by trained operators, which are expensive and require steps of extraction or sample pre treatment, increasing the time of analysis. Biosensors can overcome all these disadvantages by offering rapid, non-destructive and affordable methods for quality control. Biosensors have the potential to produce an analytical revolution to resolve the challenges in the agricultural and the food industries (Kumar, 2013)

Biosensors are defined as indicators of biological compounds that can be as simple as temperature-sensitive paint or as complex as DNA-RNA probes. Food microbiologists are constantly seeking rapid and reliable automated systems for the detection of biological activity. Biosensors provide sensitive, miniaturized systems that can be used to detect unwanted microbial activity or the presence of a biologically active compound, such as glucose or a pesticide. Immunodiagnosics and enzyme biosensors are two of the leading technologies that have had the greatest impact on the food industry. (Richter, 1993)

Biosensor devices are emerging as one of the foremost relevant diagnostic techniques for food, clinical and environmental monitoring due to their rapidity, specificity, ease of mass fabrication, economics and field applicability. They obtain their specificity from biological binding reaction, which is derived from a range of interactions that include antigen/antibody, enzyme/substrate/cofactor, receptor/ligand, chemical interactions and nucleic acid hybridization in combination with a range of transducers. It describes several applications of biosensors for food processing and safety (Ragavan, 2012)

Optical based sensing systems that measure luminescence, fluorescence, reflectance and absorbance, etc., are some of the areas of applications of optical immunosensors. Immunological methods rely on specific binding of an antibody (monoclonal, polyclonal or engineered) to an antigen. Detection of specific microorganisms and microbial toxins requires immobilization of specific antibodies onto a given transducer that can produce signal upon attachment of typical microbe/microbial toxins. Inherent features of immune sensors such as specificity, sensitivity, speed, ease and on-site analysis can be made use for various applications. Safety of food and environment has been a major concern of food technologists and health scientists in recent years. There exists a strong need for rapid and sensitive detection of different components of foods and beverages along with the food borne and water borne pathogens, toxins and pesticide residues with high specificity. Biosensors present attractive, efficient alternative techniques by providing quick and reliable performances.

There is a very good potential for application of biosensors for monitoring food quality and safety in food and bio-processing industries in India (Thakur, 2012)

Biosensors use a combination of biological receptor compounds (antibody, enzyme, nucleic acid, etc.) and the physical or physico-chemical transducer directing, in

most cases, “real-time” observation of a specific biological event (e.g. antibody–antigen interaction). They allow the detection of a broad spectrum of analytes in complex sample matrices, and have shown great promise in areas such as clinical diagnostics, food analysis, bioprocess and environmental monitoring. Ideal characteristics for a biosensor are outlined in. Biosensors may be divided into four basic groups, depending on the method of signal transduction: optical, mass, electrochemical and thermal sensors. Optical transducers are particularly attractive as they can allow direct “label-free” and “real-time detection of bacteria. Current studies focusing on optically based transduction methods aim to achieve a more robust, easy to use, portable, and inexpensive analytical system. The phenomena of surface plasmon resonance (SPR), has shown good bio sensing potential and many commercial SPR systems are now available (Kennedy, 2002).

The recent development of wireless sensor technologies and standards for wireless communications as applied to wireless sensors. Examples of wireless sensors and sensor networks applied in agriculture and food production for environmental monitoring, precision agriculture, M2M-based machine and process control, building and facility automation and RFID-based traceability systems are given. The advantages of wireless sensors and obstacles that prevent and their fast adoption (Wang, 2006).

The key benefits of biosensors including rapid and continuous measurement, high specificity, very less usage of reagents required for calibration, fast response time, ability to measure non-polar molecules that cannot be estimated by other conventional devices (Birch, 2013).

Some of the major applications of biosensors are food analysis, environmental applications, protein engineering and drug discovery applications, waste water treatment (Brian, 2013). High specificity of the biological elements used in the system. Such is the case for a number of enzymes which are able to catalyze only the reaction of a specific isomer or the extremely specific binding of antibody with antigen. Fast movement is obtained in a direct way and based on an electrical or optical signal. This also has the additional advantage that a large number of samples can be analyzed in a relatively short period of time and therefore cost can be reduced. The method is simple as sample preparation and manipulation are reduced to a minimum. Very low reagent usage as these are only necessary for calibration, maintenance of optimum conditions and, when necessary, sample dilution. The biological element can be re-used as this is usually

immobilized. On line measurements are possible, an indispensable requirement when continuous recording or automation of a process is the goal. Many processes could be rationally optimized if sensors measuring certain crucial parameters could be configured such that they permitted automatic process control. Enzyme based sensors are highly selective and fairly fast acting thus improving sensitivity by catalytic activity, Antigen/Antibody and Nucleic acids they are highly selective. They are ultra-sensitive they bind very powerfully. Tissue materials sensors have longer lifetime. Enzymes more stable as they exist in their natural environment so less subject to degradation cheaper than purified enzymes (Mathew, 2014).

III. METHODOLOGY

The methodology followed in the present study entitled “**Development of Food Sensor as an Analytical Tool in Selected Foods**” outlined in the following phases

- A. Development of Food Biosensor
- B. Place the Sensor near the Food Container/Inside the Food
- C. Detecting the Microorganism in the Selected Foods (Raw & Cooked)
- D. Monitor the Presence of Microorganism in the Selected Foods with the Reference Value
- E. Actual Value was Higher than the Reference Value, the Sensor will gives Signal to Alarm Zone.
- F. Sensor Gives Alarm through Sound or Displaying the Value.
- G. Implementation of Software.

A. DEVELOPMENT OF FOOD BIOSENSOR

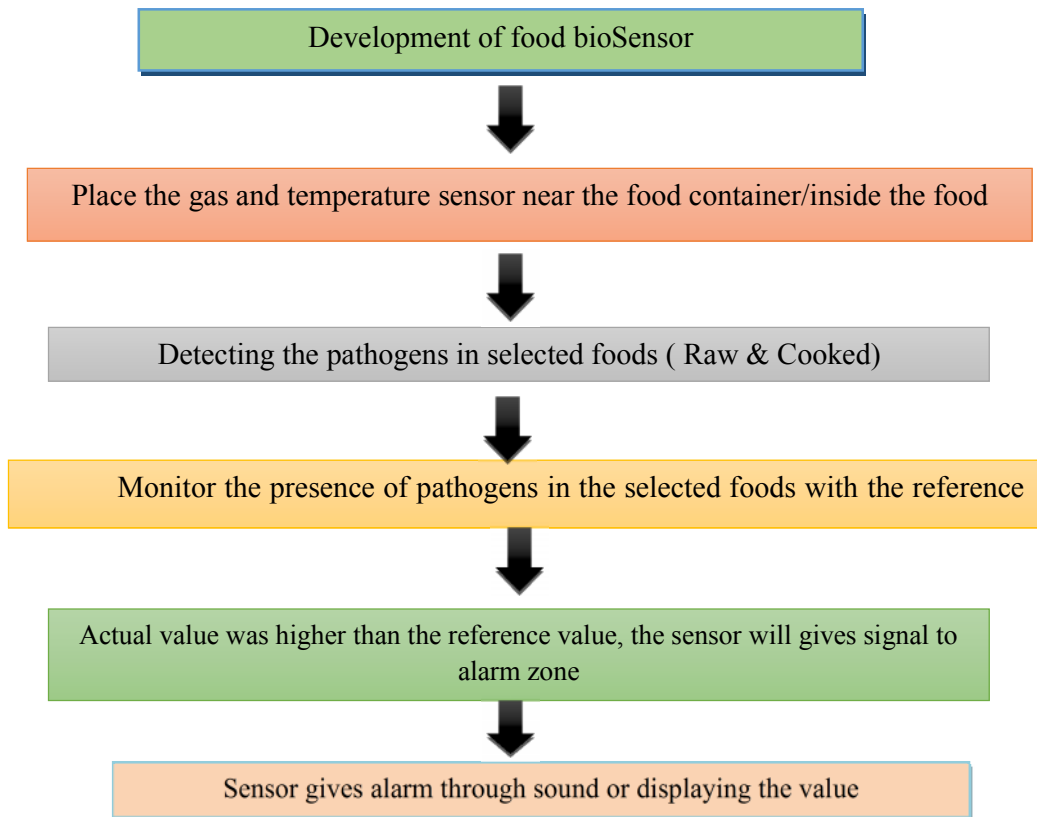


Figure 1. Development of Food Sensor

Biosensors are very important in food industry because of its rapid and continuous measurement, high specificity, very less usage of reagents required for calibration, fast response time, ability to measure non-polar molecules that cannot be estimated by other conventional devices.

BLOCK DIAGRAM

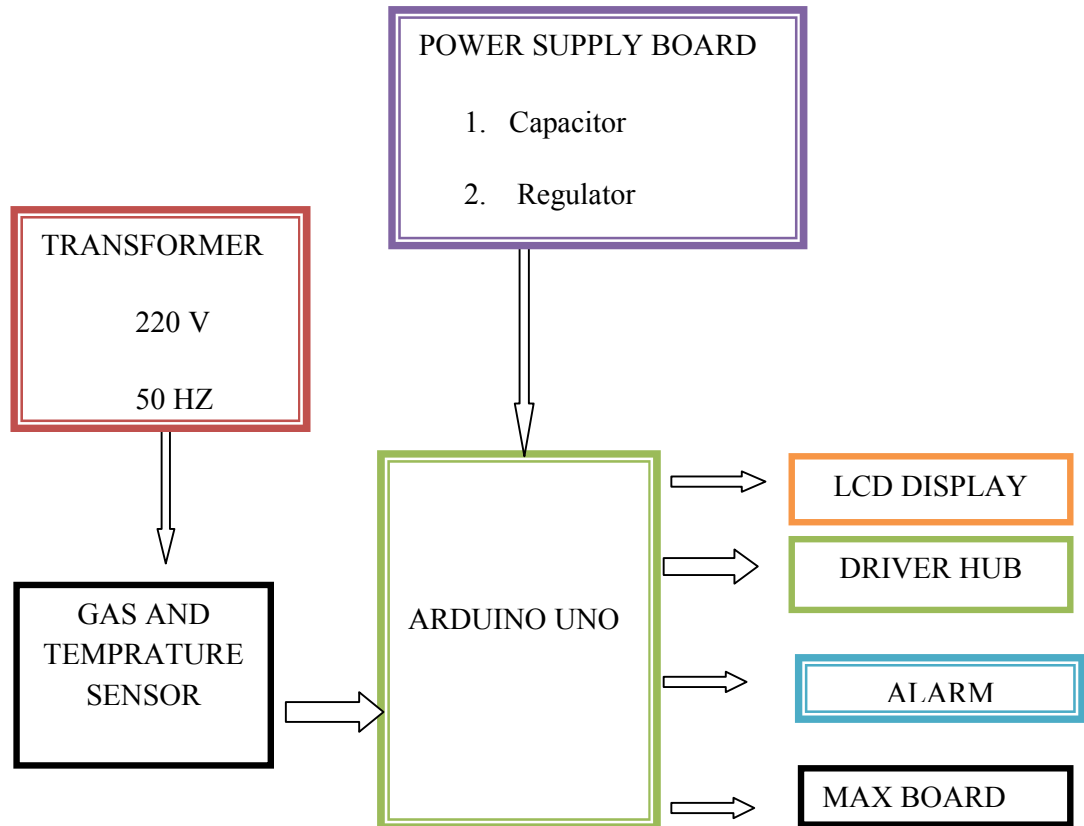


Figure 2 .Block Diagram For Sensor Development

1. GAS SENSOR

In current scenario, through the appliances like air conditioner, refrigerators are producing gases like carbon dioxide, carbon monoxide, chlorofluoro carbon etc. It is very important to monitoring the gases and monitoring of gases is very crucial in industries. Gas sensor continuously react to the gas present and it may get alters according to the molecular gaseous state. The gas module consist of steel exoskeleton under the sensing element was housed. The sensing element was subject to current through connecting leads

current is known as heating current. The gases comes sensor to the sensing element gets ionized and absorbed by the sensing element. It alters the value of the going out and changes the resistance of the sensing element. The connecting leads of the sensor are thick and it can be connected firmly to the circuit through that sufficient amount of heat gets conducted to the inside parts. They are casted from copper and have tin plating over them. This unit will be incorporated easily into a alarm zone. It gives the signals through sound or displaying the visual indication of a gas concentration. The sensor has high sensitive and its response quickly



Figure 3. Gas Sensor

2.CIRCUIT DISCRIPTION

For the present study circuit was mainly designed to sense the level of gas present in the selected food particles. The sensor is a type of transducer, which produces the voltage signal; it depends up on the presence of gas level. The voltage signal is given to the inverting input terminal of the comparator. The comparator is constructed by the operational amplifier, it compares the normal reference signal and it produces the output error signal. The microcontroller receives the output voltage to determine the presence of effective gas (Doll, 2003)

3. TEMPRATURE SENSOR

Temperature sensor is a device which senses the various temperatures. LM35 is a basic temperature sensor. It is used for the experimental studies and purposes. It gives the reading in centigrade or degree Celsius. A digital temperature sensor provides 9 – bit temperature reading. It gives an accurate reading. The sensor is designed to read the value from 0° to 70° C. It is possible to read $\pm 0.5^\circ$ C. occasionally. The digital temperature sensor consist of three terminals and it requires maximum 5.5 voltage supply. This type of

Liquid crystal display is a thin, flat electronic visual display. It uses the light modulating properties of a liquid crystals. LCD contains passive display technology so it does not emit the light directly. The interface between LCD and microcontroller is 8 bit or 4 bit. It varies according to the data or commands are receiving by a LCD. 8 bit receives data or commands through data lines DBO – DB7 and data strobe, the input is given to the LCD. 4 bit mode uses only 4 data lines and 8 bit mode are splitted into two parts (each parts contain 4 bits) data and commands are received through the DBO. DB7 and its own data strobe through the input. These 4 bit are used to save the microcontroller pins. The speed difference between 4 bit and 8 bit modes are minimal (Chen, 2011)

5. ARDUINO UNO

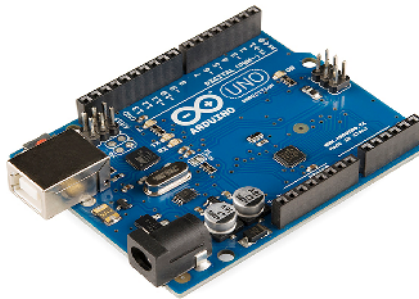


Figure 6 Arduino Uno

Arduino Uno is a type of microcontroller board which works like CPU in the PC. It contain 14 digital input/output pins (6 can be used for PWM outputs), 6 analog inputs, a 16 MHz crystal oscillator, a USB connection, a power jack, an ICSP header and reset button. The devices like Sensor, Bluetooth, WI FI modules are connected with arduino UNO. Based on the coding the arduino UNO will work properly. It contains everything which is needed to support the microcontroller, Arduino Uno will be connect via USB connection or with an external power supply. The power source will selected automatically. External (non-USB) power can come through AC-to-DC adapter or battery. The adapter should be connected by using plugging 2.1mm centre-positive plug into the board's power jack. Through power connector header pin (VIN & GND) leads from the battery will be inserted. 6 to 20 volts will be supplied by externally to operate the boards. If the supply is less than 7V, the 5V pin may get supply less than five volts and the board will be unstable. If we use more than 12V, the voltage regulator will overheat and damage the board. The recommended range is between 7 to 12 volts. The UNO contain 6 analog

inputs, each of these will provide 10 bits of resolution (i.e. 1024 different values). By its default it will measure from the ground to 5 volts, though it is possible to change upper end of their range using the AREF pin and the analog reference function and some pins have their specialized function (Barrett., 2012).

6. DRIVER HUB

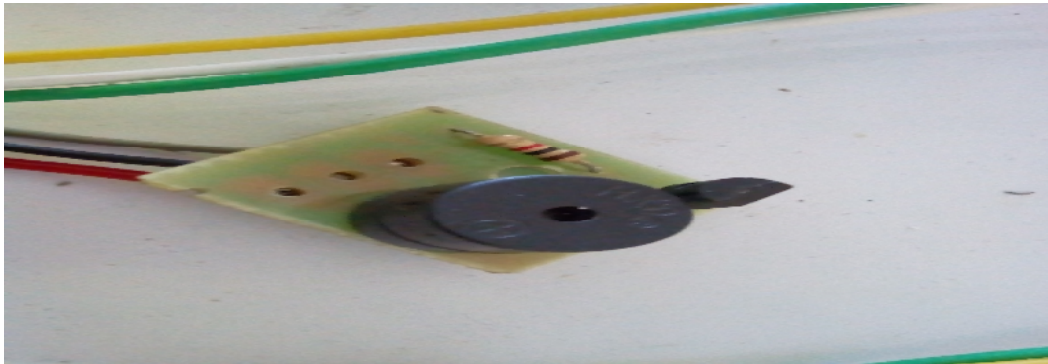


Figure 7 Driver Hub

Driver hub is an electronic device. It performs as an tool in that device, the driver hub installer is less than 1 Mb size. It used to scan the system using out data drivers. The application will find display drivers, audio, sound card drivers, graphics and video drivers, scanner driver, mother board drivers and so on. The latest drive is used mostly in hardware. Driver hub is helpful for setting alarm, switch on and off button (Krems, 2013)

7. ALARM

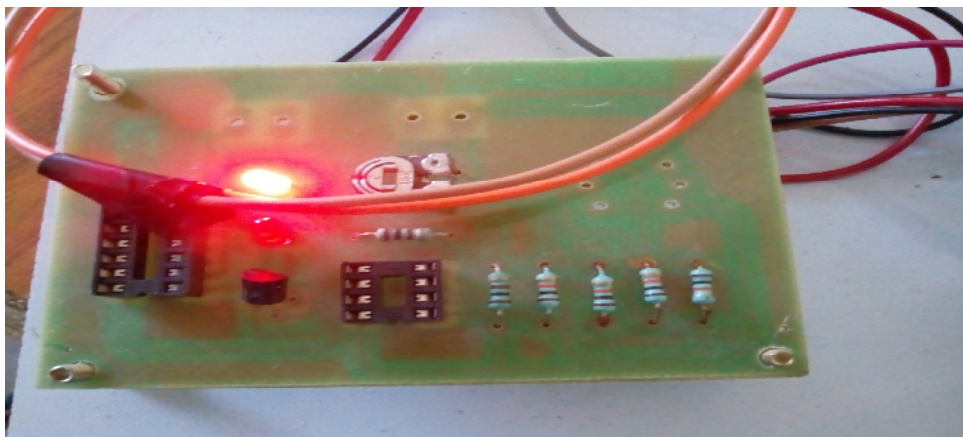


Figure 8 Alarm

Alarm is a part of sensor board. It receives the signal from the microcontroller and it gives result through display or sound. Frequency and band width can be used to receive signals from microcontroller. It works according to some topology, it display a same messages more times. There are two types of topology, they are star and bus topology (Pretty,2013)

8. TRANSFORMER

Current Transformers produce an output in proportion to the current flowing through the primary winding as a result of a constant potential on the primary. The Current Transformer (C.T) is a type of “instrument transformer” it is designed to produce an alternating current in its secondary winding which is proportional to the current being measured in its primary. Current transformers reduce high voltage currents to a much lower value and provide a convenient way of safely monitoring the actual electrical current flowing in an AC transmission line using a standard ammeter. The principal of operation of a basic current transformer is slightly different from that of an ordinary voltage transformer. This primary winding can be of either a single flat turn, a coil of heavy duty wire wrapped around the core or just a conductor or bus bar placed through a central hole as shown. Due to this type of arrangement, the current transformer is often referred too as a “series transformer” as the primary winding, which never has more than a very few turns, is in series with the current carrying conductor supplying a load. The secondary winding however, may have a large number of coil turns wound on a laminated core of low-loss magnetic material. This core has a large cross sectional area so that the magnetic flux density created is low using much smaller cross sectional area wire, depending upon how much the current must be stepped down as it tries to output a constant current, independent of the connected load. The secondary winding will supply a current into either a short circuit, in the form of an ammeter, or into a resistive load until the voltage induced in the secondary is big enough to saturate the core or cause failure from excessive voltage breakdown. The transformer input is 230 V and 50 HZ. It will step down to 9 V and it gives as a AC voltage(Harlow, 2004)

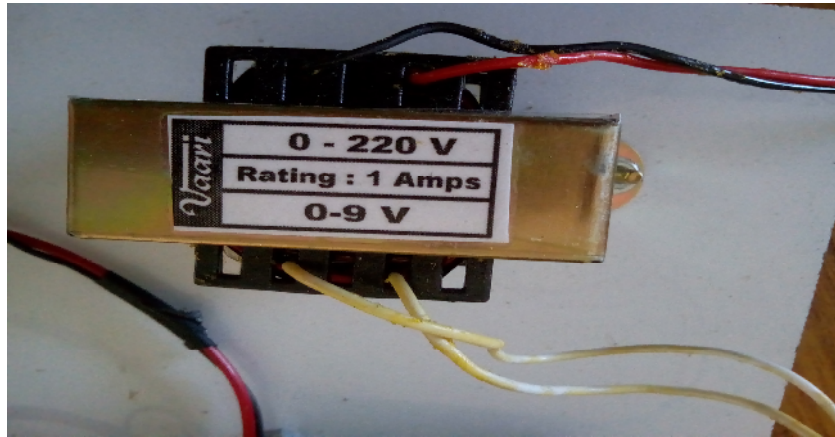


Figure 9 Transformer

9. POWER SUPPLY BOARD

Through the power supply board, the power will supply to the sensor circuit board. It will convert AC voltage to DC voltage and it is also known as bridge rectifier. It consist of two components

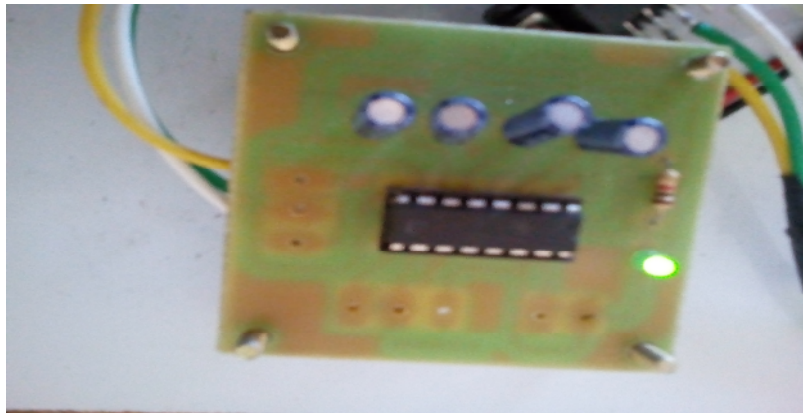


Figure 10 Power Supply Board

10. CAPACITOR

The converted DC Voltage can't supply properly to the sensor circuit. So capacitor is used to filter the DC voltage current.

11. REGULATOR

The circuit system needed only 5V for its proper functioning. The regulator will give the accurate 5 voltage

12. MAX BOARD

The PC will work only on 12 Voltage to 19 Voltage, through help of this PC will send and receive the data and information to the microcontroller and to the PC (Gurevich, 2017)

B. PLACE THE SENSOR NEAR THE FOOD CONTAINER/INSIDE THE FOOD

Place the temperature and gas sensor downwards to the food sample. It will sense and monitor the gas and temperature values and send the value to the micro controller.

C. DETECTING THE MICROORGANISM IN THE SELECTED FOODS (RAW & COOKED)

Researcher have to check the food before and after cooking (1/2hr, 1hr, 1 ½ hr, 2hr.) for its microbial contamination and its spoilage level using through the gas and temperature sensor.

Milk, water, fruit juices, fermented rice, idli batter, butter, cheese, curds and fleshy foods are the food commodities were selected as sample because these foods are commonly used by everyone and also it can be contaminated by the pathogens very easily causes food borne diseases and food poisoning because of the production of ethylene gas, so these foods were selected.

D. MONITOR THE PRESENCE OF MICROORGANISM IN THE SELECTED FOODS WITH THE REFERENCE VALUE

Selected foods like milk, water, fruit juices, fermented rice, idli batter, butter, cheese, curds and fleshy foods were monitored periodically after ½ hour, 1 hour, 1 ½ hour and 2 hours and check for the presence of microorganism. The selected food commodities will be sensed by using developed sensor circuit.

E. SENSOR CHECKS AND COMPARE WITH THE REFERENCE VALUE

The sensor will sense the food and the value will be displayed in the serial monitor, the data displayed in the serial monitor will be read and compared with the reference value. If actual value was higher than the reference value, the sensor will give signal to alarm zone. If the real value is higher than the reference value, the sensor gives signals through microcontroller to the alarm zone.

F. SENSOR GIVES ALARM THROUGH SOUND OR DISPLAYING THE VALUE

Through microcontroller, alarm zone receives the signal. When the researcher connect speaker with arduino UNO while the alarm is ringing it gives the signal through the sound or the value will display through liquid crystal display (LCD).

G. IMPLEMENTATION OF SOFTWARE.

1. Implementation of Arduino Uno

PLATE 1: Starting the Arduino Uno software



PLATE 2 : Type the Coding (coding is given in the appendix)

```
Menu | Arduino 1.8.5
File Edit Sketch Tools Help
Main
#include <LiquidCrystal.h>

LiquidCrystal lcd (7,6,5,4,3,2);
int alarm =8;
void LCD_Decimal3(unsigned int val);

void serial_Decimal3(unsigned char val);

unsigned int Temp, Gas, num, movee, k, Mic count, server time;
char m, val [10];

void setup()
{
  lcd.begin(16, 2);
  Serial.begin(9600);

  pinMode(A0, INPUT);
  pinMode(A1, INPUT);
  pinMode(alarm, OUTPUT);

  /* lcd.setCursor(0, 0);
  lcd.print(" WEARABLE ");
  lcd.setCursor(0, 1);
  lcd.print(" TEXTILE ");
```

PLATE 3: Verify the Typed Coding

```
Menu | Arduino 1.8.5
File Edit Sketch Tools Help
Main
#include <LiquidCrystal.h>

LiquidCrystal lcd (7,6,5,4,3,2);
int alarm =8;
void LCD_Decimal3(unsigned int val);

void serial_Decimal3(unsigned char val);

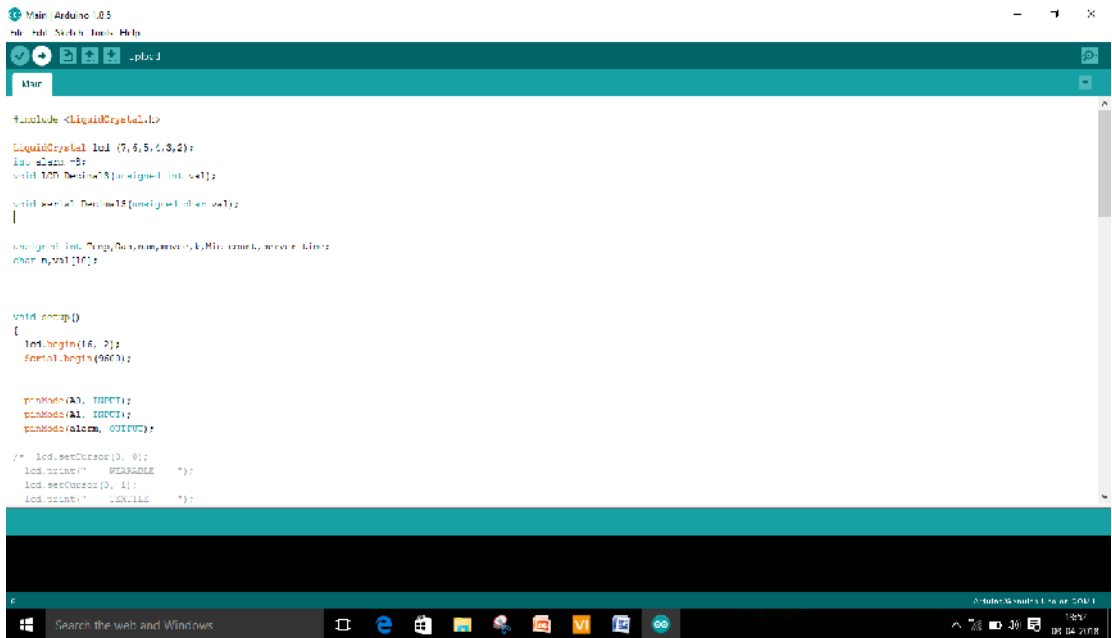
unsigned int Temp, Gas, num, movee, k, Mic count, server time;
char m, val [10];

void setup()
{
  lcd.begin(16, 2);
  Serial.begin(9600);

  pinMode(A0, INPUT);
  pinMode(A1, INPUT);
  pinMode(alarm, OUTPUT);

  /* lcd.setCursor(0, 0);
  lcd.print(" WEARABLE ");
  lcd.setCursor(0, 1);
  lcd.print(" TEXTILE ");
```

PLATE 4: Upload the Verified Coding to Arduino Uno Microcontroller



```

Main | Arduino 1.0.5
File Edit Tools Help
- - - - -
Altair

#include <LiquidCrystal.h>

LiquidCrystal lcd( 7, 6, 5, 4, 3, 2);
int alarm = 8;
void lcdWrite8(unsigned int val);
void lcdWrite16(unsigned int val);

unsigned int Temp, Gas, man, pres, s, M, i, r, m, t, w, v, e, r, t;
char n_val[10];

void setup()
{
  lcd.begin(16, 2);
  Serial.begin(9600);

  pinMode(A3, INPUT);
  pinMode(A1, INPUT);
  pinMode(alarm, OUTPUT);
}

/* lcd.setCursor(0, 0);
   lcd.print(" WELCOME ");
   lcd.setCursor(0, 1);
   lcd.print(" WELCOME ");
*/

```

2. IMPLEMENTATION OF LABVIEW

PLATE 5 : Starting the LabView Tools Software



PLATE 6 :Front Page of the Software

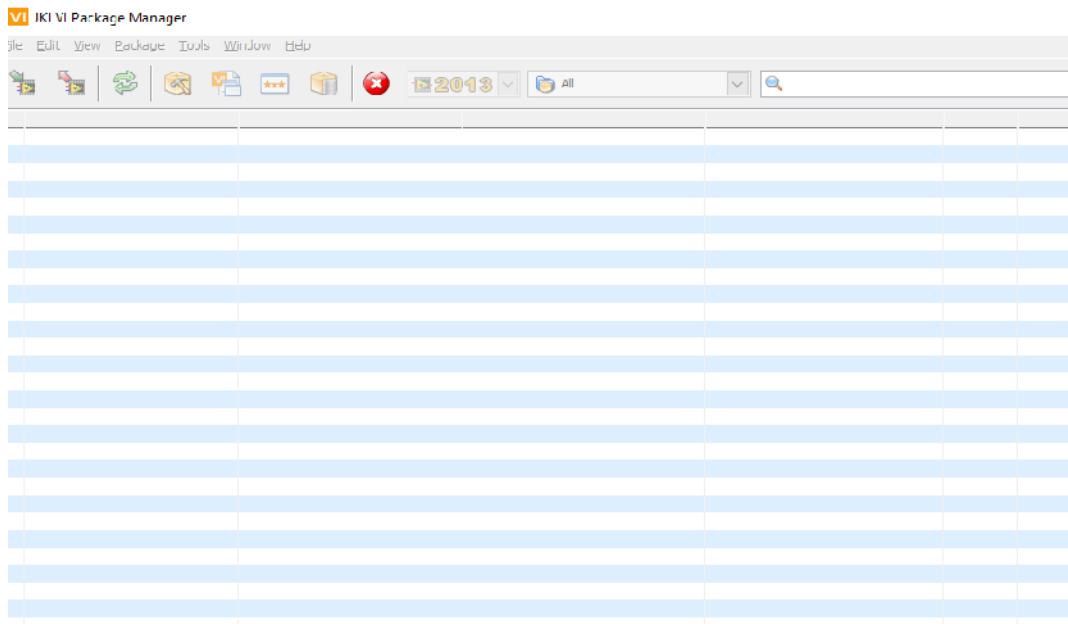


PLATE 7 :Designing the Front Panel of the Software

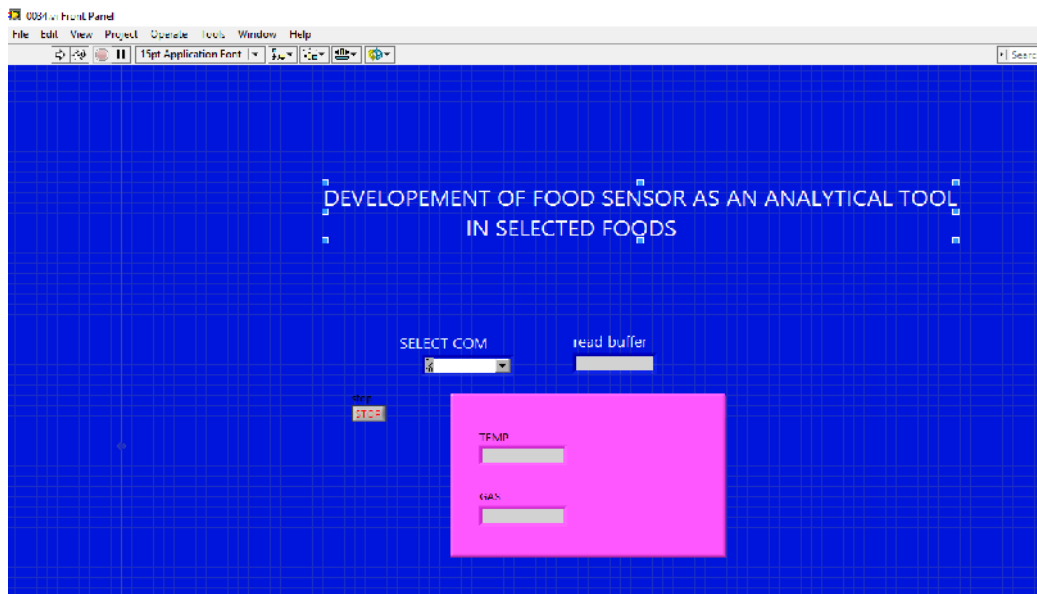


PLATE 8: Creating the Block Diagram of the Circuit Board

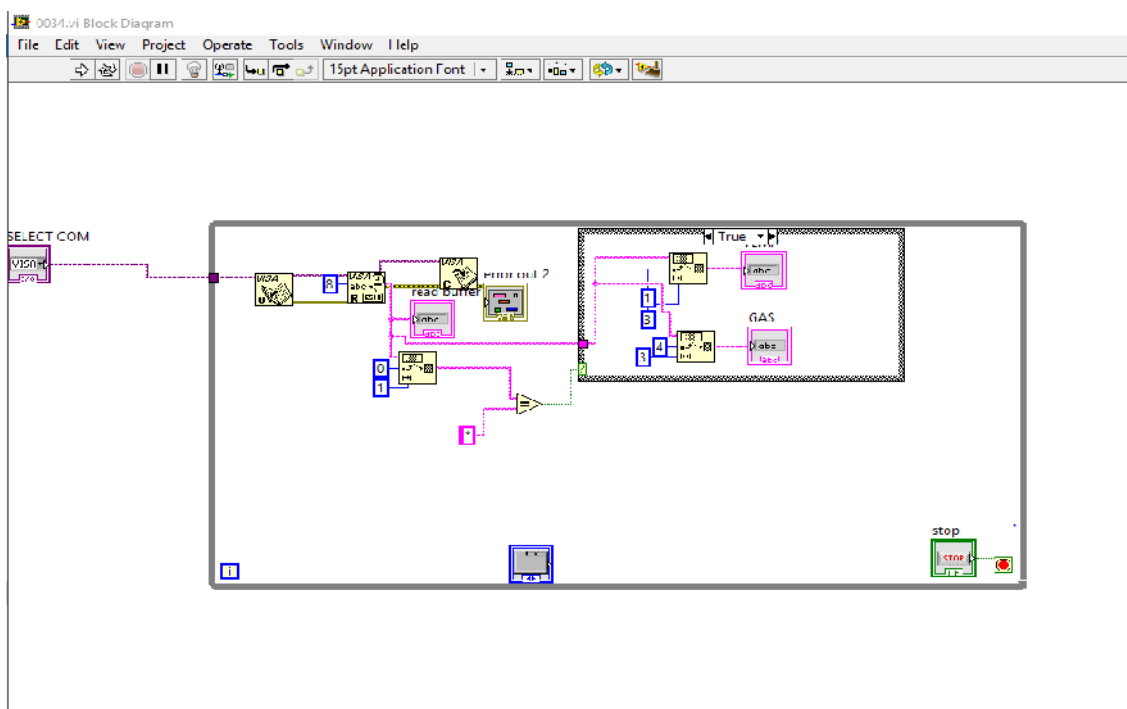
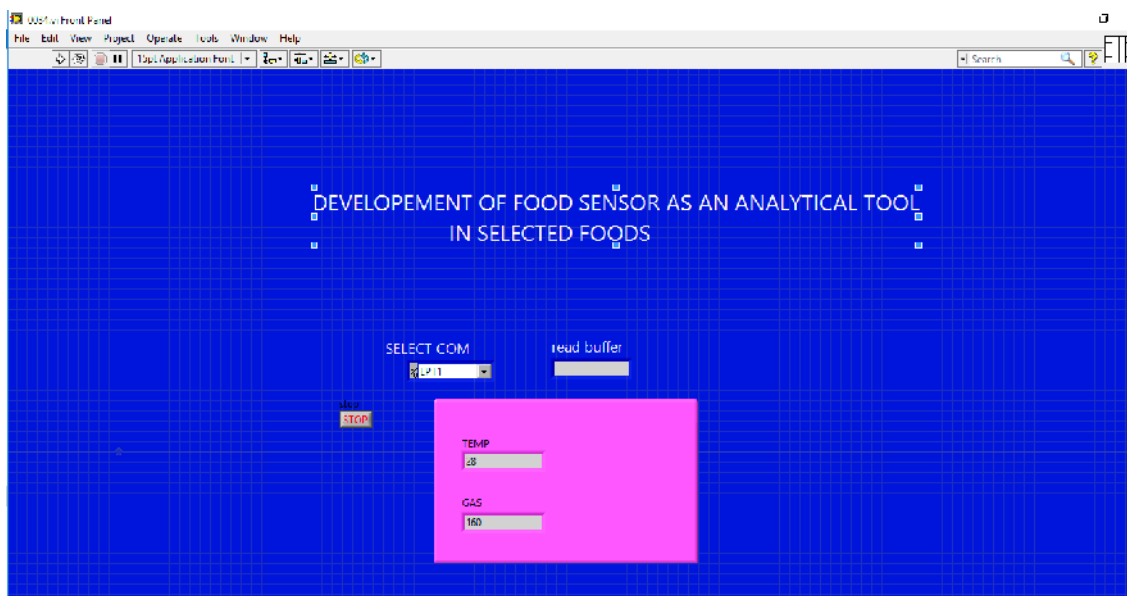


PLATE 9 : Running the Output



The present study was approved by the *INSTITUTIONAL HUMAN ETHICAL COMMITTEE* Avinashilingam Institute for Home Science for Women, Coimbatore on 19th MARCH, 2018. **Approval No is A UW/IHEC/FSMD-17-18/XPD/06.**

IV. RESULTS AND DISCUSSION

The result and discussion pertaining to the present study “**Development of Food Sensor as an Analytical Tool in Selected Foods**” under the following headings

- A. Hardware Implementation.
- B. Experimental Output

A. Hardware Implementation

1. Power supply

Block Diagram



Figure 11. Block diagram for Power supply

The ac voltage, typically 220V rms, is connected to a transformer, which steps that AC voltage down to the level of the desired DC output. A diode rectifier then provides a full-wave rectified voltage that is initially filtered by a simple capacitor filter to produce a DC voltage. This resulting DC voltage usually has some ripple or AC voltage variation.

A regulator circuit removes the ripples and also remains the same DC value even if the input DC voltage varies. This voltage regulation is usually obtained using one of the popular voltage regulator IC units.

Schematic Diagram

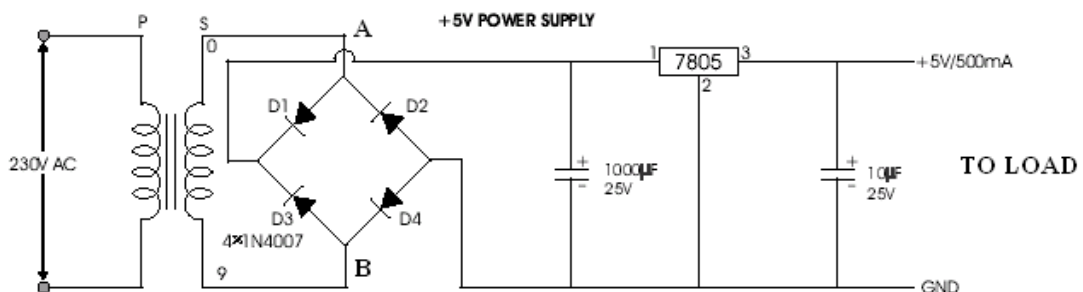


Figure 12. Schematic Diagram Power Supply

2. Working Principle

a. Transformer

The potential transformer will step down the power supply voltage (0-230V) to (0-9V) level. If the secondary has less turns in the coil than the primary, the secondary coil's voltage will decrease and the current or AMPS will increase or decreased depend upon the wire gauge. **This is called a STEP-DOWN transformer.** Then the secondary of the potential transformer will be connected to the rectifier.

b. Bridge Rectifier

When four diodes are connected as shown in figure, the circuit is called as bridge rectifier. The input to the circuit is applied to the diagonally opposite corners of the network, and the output is taken from the remaining two corners.

Let us assume that the transformer is working properly and there is a positive potential, at point A and a negative potential at point B. the positive potential at point A will forward bias D3 and reverse bias D4.

The negative potential at point B will forward bias D1 and reverse D2. At this time D3 and D1 are forward biased and will allow current flow to pass through them; D4 and D2 are reverse biased and will block current flow.

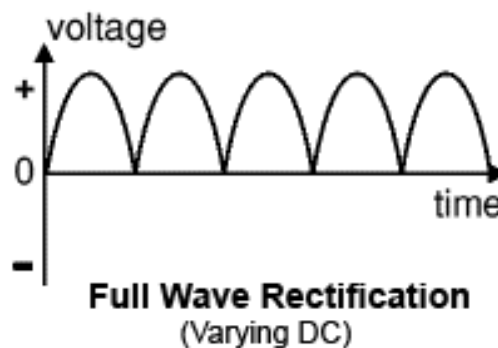


Figure 13 Full wave Rectification

The path for current flow is from point B through D1, up through Load, through D3, through the secondary of the transformer back to point B.

One-half cycle later the polarity across the secondary of the transformer reverse, forward biasing D2 and D4 and reverse biasing D1 and D3. Current flow will now be from point A through D4, up through Load, through D2, through the secondary of transformer, and back to point A. Across D2 and D4. The current flow through Load is always in the same direction. In flowing through Load this current develops a voltage corresponding to that. Since current flows through the load during both half cycles of the applied voltage, this bridge rectifier is a full-wave rectifier.

One advantage of a bridge rectifier over a conventional full-wave rectifier is that with a given transformer the bridge rectifier produces a voltage output that is nearly twice that of the conventional half-wave circuit.

This bridge rectifier always drops 1.4Volt of the input voltage because of the diode. We are using 1N4007 PN junction diode, its cut off region is 0.7Volt. So any two diodes are always conducting, total drop voltage is 1.4 volt.

c. Filter

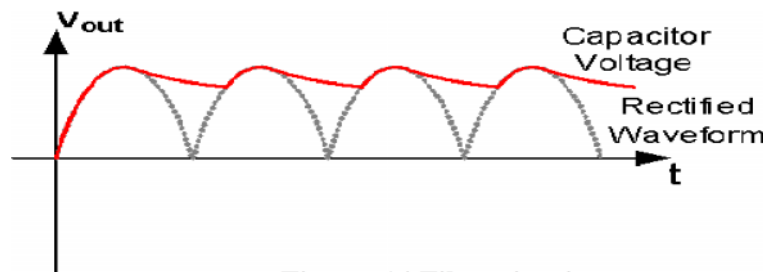


Figure 14 Filter circuit

If a Capacitor is added in parallel with the load resistor of a Rectifier to form a simple Filter Circuit, the output of the Rectifier will be transformed into a more stable DC Voltage. At first, the capacitor is charged to the peak value of the rectified Waveform. Beyond the peak, the capacitor is discharged through the load until the time at which the rectified voltage exceeds the capacitor voltage. Then the capacitor is charged again and the process repeats itself.

d. IC Voltage Regulators

Voltage regulators comprise a class of widely used ICs. Regulator IC units contain the circuitry for reference source, comparator amplifier, control device, and overload

protection all in a single IC. IC units provide regulation of either a fixed positive voltage, a fixed negative voltage, or an adjustably set voltage.

The series 78 regulators provide fixed positive regulated voltages from 5 to 24 volts. Similarly, the series 79 regulators provide fixed negative regulated voltages from 5 to 24 volts.

This is a regulated power supply circuit using the 78xx IC series. These regulators can deliver current around 1A to 1.5A at a fix voltage levels. The common regulated voltages are 5V, 6V, 8V, 9V, 10V, 12V, 15V, 18V, and 24V. It is important to add capacitors across the input and output of the regulator IC to improve the regulation.

In this circuit we are using 7805 regulator so it converts variable dc into constant positive 5V power supply. If the input voltage goes to below 7.3Volt means the output also varied. That is why we are using 230/9V step-down transformer. Transformer output is higher than the regulator minimum level input.

e. Temperature Sensors

This sensor will measure the resistance against temperature of a thermistor. It will check how well it follows a given theoretical pattern. It will consider the use of the thermistor in a potential divider as a temperature sensor. The Temperature Sensor will consider the design of a remote sensor. It will find out how thermistors work.

Apparatus

- 1 Disc thermistor (RS 256-102) with leads
- 2 Beakers
- Digital multimeter
- 1 Thermometer (0 0C - 100 0C)
- Access to a kettle of hot water
- Access to iced water

3. Microcontroller Process

The Microcontrollers work between the Enter your data into Graphical Analysis or a similar program and use its curve-fitting facilities to see how well the equation matches

your experiment. Include a copy of the graph in your report. Comment below on the quality of the fit.

The potential divider circuits used for sensors. One thing you discovered was that the circuit would be most sensitive if the resistor's value matched that of the sensor in its working conditions. Suppose you intend to use the thermistor from this experiment in a potential divider circuit as shown on the right. It is intended to monitor human body temperature (around 37 °C).

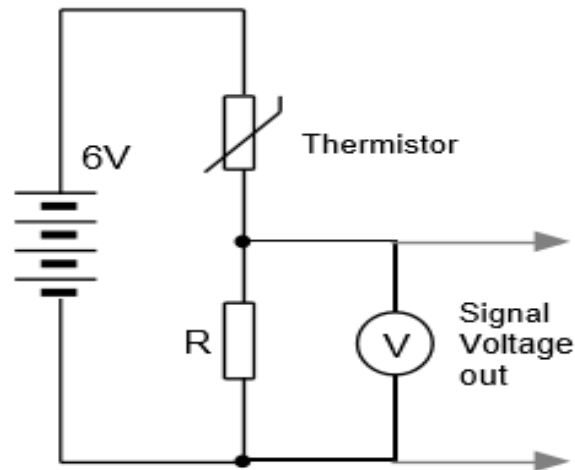


Figure.15 Circuit diagram of Temperature sensor

4. Thermistors as Temperature Sensors

The Temperature sensors food based electronic devices it can be used input thermistor and the output is given as the signal voltage output. The finally in this research thermistors work it can be food based change the temperature Sensor and the microcontroller wave connected the output.

5. Electrochemical Gas Sensors

Electrochemical gas sensors are ethylene gas detectors that measure the concentration of a target gas by oxidizing or reducing the target gas at an electrode and measuring the resulting current. This unit can be easily incorporated into an alarm unit, to sound an alarm or give a visual indication of the ethylene gas concentration. The sensor has excellent sensitivity combined with a quick response time. The sensor can also sense iso-butane, propane and LNG.

Features

- High Sensitivity
- Detection Range: 100 - 10,000 ppm iso-butane propane
- Fast Response Time: <10s
- Heater Voltage: 5.0V
- Dimensions: 18mm Diameter, 17mm High excluding pins, Pins - 6mm High

6. Microcontroller Process

The gas diffuses into the sensor, through the back of the porous membrane to the working electrode where it is oxidized or reduced. This electrochemical reaction results in an electric current that passes through the external circuit. In addition to measuring, amplifying and performing other signal processing functions, the external circuit maintains the voltage across the sensor between the working and counter electrodes for a two electrode sensor or between the working and reference electrodes for a three electrode cell. At the counter electrode an equal and opposite reaction occurs, such that if the working electrode is an oxidation, then the counter electrode is a reduction.

7. Cross Sensitivity

For some gases such as ethylene oxide, cross sensitivity can be a problem because ethylene oxide requires a very active working electrode catalyst and high operating potential for its oxidation. Therefore gases which are more easily oxidized such as alcohols and carbon monoxide will also give a response. Cross sensitivity problems can be eliminated though through the use of a chemical filter, filters that allows the target gas to pass through unimpeded, but which reacts with and removes common interferences.

While electrochemical sensors offer many advantages, they are not suitable for every gas. Since the detection mechanism involves the oxidation or reduction of the gas, electrochemical sensors are usually only suitable for gases which are electrochemically active, though it is possible to detect electrochemically inert gases indirectly if the gas interacts with another species in the sensor that then produces a response.^[2] Sensors for carbon dioxide are an example of this approach and they have been commercially available for several years.

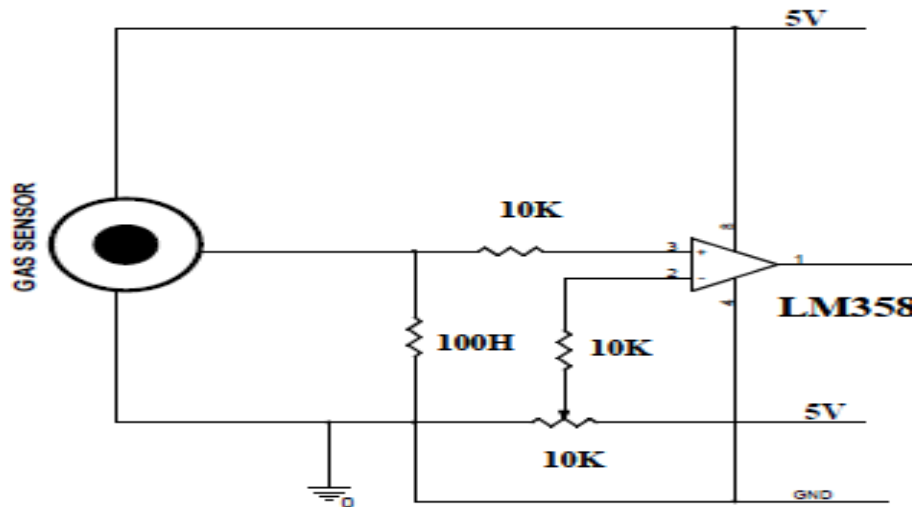


Figure 16. Cross Sensitivity of Gas Sensor

8. Circuit Description

The gas sensor is the special sensor which designed for sense the gas leakage. In the gas sensor the supply voltage is given to input terminal. The gas sensor output terminals are connected to non inverting input terminal of the non inverting amplifier. Non inverting amplifier amplifies the voltage level and it will given to the pic microcontroller.

9. Arduino

The ATmega328P is a low-power CMOS 8-bit microcontroller based on the AVR enhanced RISC architecture. By executing powerful instructions in a single clock cycle, the ATmega328P achieves throughputs approaching 1 MIPS per MHz allowing the system designer to optimize power consumption versus processing speed.

10. Pin Details

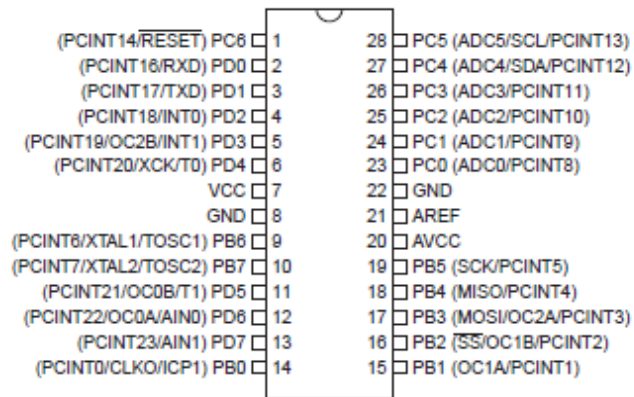


Figure17 .Pin Details of Arduino Uno

a. Pin Descriptions

VCC

Digital supply voltage.

GND

Ground.

Port B (PB7:0) XTAL1/XTAL2/TOSC1/TOSC2

Port B is an 8-bit bi-directional I/O port with internal pull-up resistors (selected for each bit). The Port B output buffers have symmetrical drive characteristics with both high sink and source capability. As inputs, Port B pins that are externally pulled low will source current if the pull-up resistors are activated. The Port B pins are tri-stated when a reset condition becomes active.

If the Internal Calibrated RC Oscillator is used as chip clock source, PB7..6 is used as TOSC2..1 input for the Asynchronous Timer/Counter2 if the AS2 bit in ASSR is set.

PC6/RESET

If the RSTDISBL Fuse is programmed, PC6 is used as an I/O pin. Note that the electrical characteristics of PC6 differ from those of the other pins of Port C. If the RSTDISBL Fuse is unprogrammed, PC6 is used as a Reset input. A low level on this pin for longer than the minimum pulse length will generate a Reset, even if the clock is not running.

11. AVCC

AVCC is the supply voltage pin for the A/D Converter, PC3:0, and ADC7:6. It should be externally connected to VCC, even if the ADC is not used. If the ADC is used, it should be connected to VCC through a low-pass filter. Note that PC6..4 use digital supply voltage, VCC.

BLOCK DIAGRAM

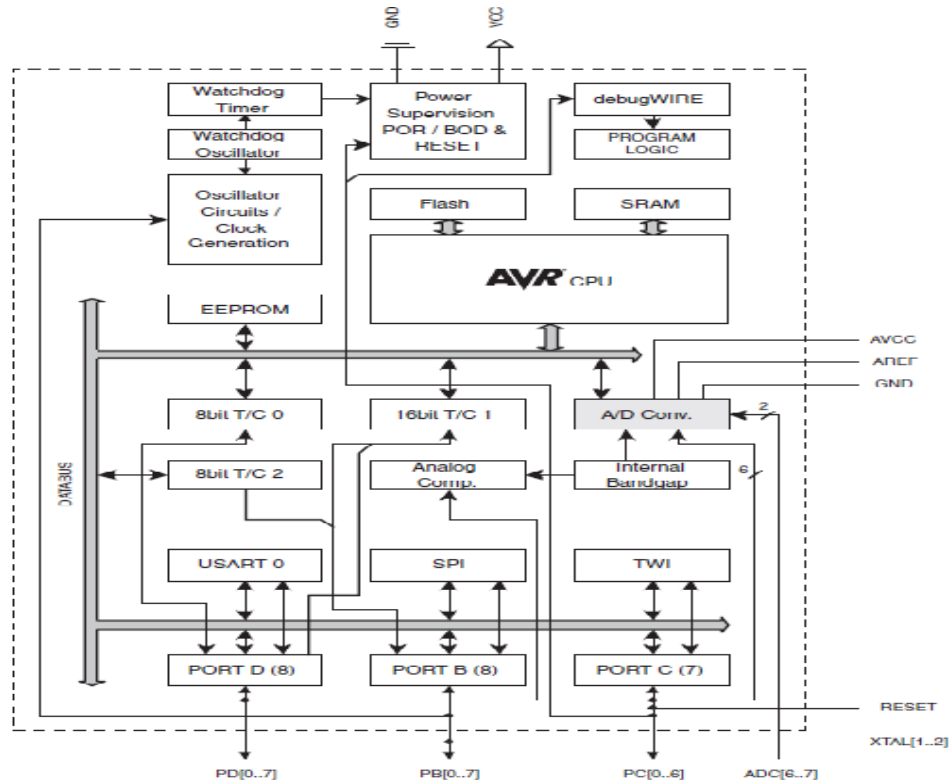


Figure 18. Block Diagram of AVCC

The AVR core combines a rich instruction set with 32 general purpose working registers. All the 32 registers are directly connected to the Arithmetic Logic Unit (ALU), allowing two independent registers to be accessed in one single instruction executed in one clock cycle. The resulting architecture is more code efficient while achieving throughputs up to ten times faster than conventional CISC microcontrollers.

The ATmega328P AVR is supported with a full suite of program and system development tools including: C Compilers, Macro Assemblers, Program Debugger/Simulators, In-Circuit Emulators, and Evaluation kits.

12.AVR CPU Core

Overview

This section discusses the AVR core architecture in general. The main function of the CPU core is to ensure correct program execution. The CPU must therefore be able to access memories, perform calculations, control peripherals, and handle interrupts.

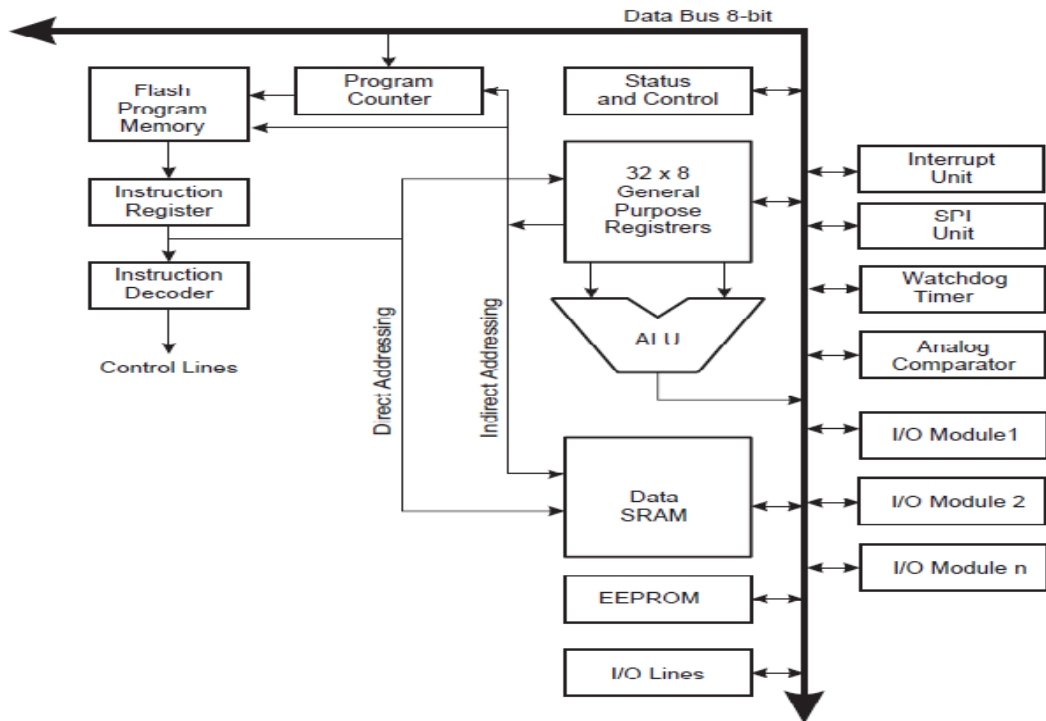


Figure 19. Overview of AVR Core

The interrupts and subroutine calls, the return address Program Counter (PC) is stored on the Stack. The Stack is effectively allocated in the general data SRAM, and consequently the Stack size is only limited by the total SRAM size and the usage of the SRAM. All user programs must initialize the SP in the Reset routine (before subroutines or interrupts are executed). The Stack Pointer (SP) is read/write accessible in the I/O space. The data SRAM can easily be accessed through the five different addressing modes supported in the AVR architecture. The memory spaces in the AVR architecture are all linear and regular memory maps. The lower the Interrupt Vector address, the higher the priority.

The I/O memory space contains 64 addresses for CPU peripheral functions as Control Registers, SPI, and other I/O functions. The I/O Memory can be accessed directly,

or as the Data Space locations following those of the Register File, 0x20 - 0x5F. In addition, the ATmega328P has Extended I/O space from 0x60 - 0xFF in SRAM where only the ST/STS/STD and LD/LDS/LDD instructions can be used.

13. ALU – Arithmetic Logic Unit

The high-performance AVR ALU operates in direct connection with all the 32 general purpose working registers. Within a single clock cycle, arithmetic operations between general purpose registers or between a register and an immediate are executed. The ALU operations are divided into three main categories – arithmetic, logical, and bit-functions. Some implementations of the architecture also provide a powerful multiplier supporting both signed/unsigned multiplication and fractional format. See the “Instruction Set” section for a detailed description.

14. Status Register

The Status Register contains information about the result of the most recently executed arithmetic instruction. This information can be used for altering program flow in order to perform conditional operations. Note that the Status Register is updated after all ALU operations, as specified in the Instruction Set Reference.

Bit 7 – I: Global Interrupt Enable

The Global Interrupt Enable bit must be set for the interrupts to be enabled. The individual interrupt enable control is then performed in separate control registers. If the Global Interrupt Enable Register is cleared, none of the interrupts are enabled independent of the individual interrupt enable settings.

Bit 6 – T: Bit Copy Storage

The Bit Copy instructions BLD (Bit Load) and BST (Bit Store) use the T-bit as source or destination for the operated bit. A bit from a register in the Register File can be copied into T by the BST instruction, and a bit in T can be copied into a bit in a register in the Register File by the BLD instruction.

Bit 5 – H: Half Carry Flag

The Half Carry Flag H indicates a Half Carry in some arithmetic operations. Half carry is useful in BCD arithmetic.

Bit 4 – S: Sign Bit, S = N ⊕ V

The S-bit is always an exclusive or between the Negative Flag N and the Two's Complement Overflow Flag V.

Bit 3 – V: Two's Complement Overflow Flag

The Two's Complement Overflow Flag V supports two's complement arithmetics.

Bit 2 – N: Negative Flag

The Negative Flag N indicates a negative result in an arithmetic or logic operation.

Bit 1 – Z: Zero Flag

The Zero Flag Z indicates a zero result in an arithmetic or logic operation.

Bit 0 – C: Carry Flag

The Carry Flag C indicates a carry in an arithmetic or logic operation

15.General Purpose Register File

The Register File is optimized for the AVR Enhanced RISC instruction set. In order to achieve the required performance and flexibility, the following input/output schemes are supported by the Register File

- One 8-bit output operand and one 8-bit result input
- Two 8-bit output operands and one 8-bit result input
- Two 8-bit output operands and one 16-bit result input
- One 16-bit output operand and one 16-bit result input

16.Stack Pointer

The Stack is mainly used for storing temporary data, for storing local variables and for storing return addresses after interrupts and subroutine calls. Note that the stack is implemented as growing from higher to lower memory locations. The Stack Pointer Register always points to the top of the Stack.

17.Instruction Execution Timing

This section describes the general access timing concepts for instruction execution. The AVR CPU is driven by the CPU clock clk CPU, directly generated from the selected clock source for the chip. No internal clock division is used. The figure shows the parallel

instruction fetches and instruction executions enabled by the Harvard architecture and the fast-access Register File concept. This is the basic pipelining concept to obtain up to 1 MIPS per MHz with the corresponding unique results for functions per cost, functions per clocks, and functions per power-unit.

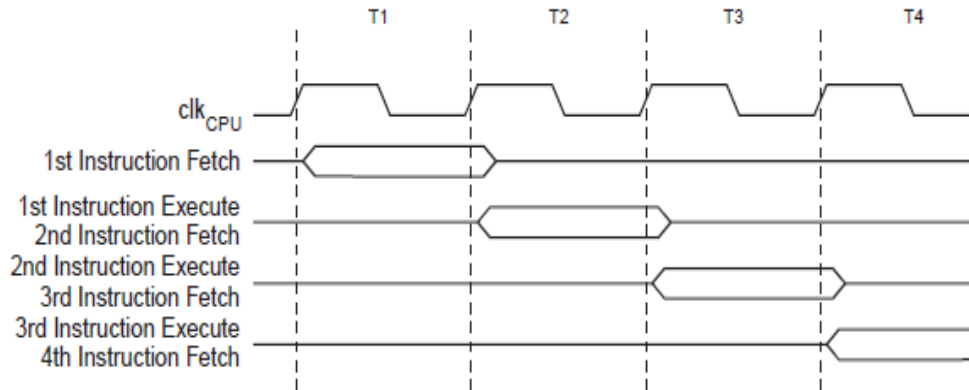


Figure 20. Parallel Instruction Fetches and Instruction Executions

The below figure shows the internal timing concept for the Register File. In a single clock cycle an ALU operation using two register operands is executed, and the result is stored back to the destination register.

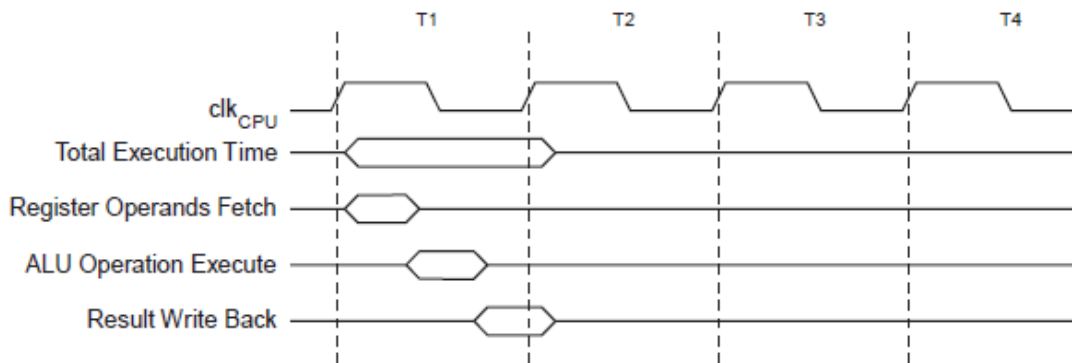


Figure 21. Single Cycle ALU Operation

18. Reset and Interrupt Handling

The AVR provides several different interrupt sources. These interrupts and the separate Reset Vector each have a separate program vector in the program memory space. All interrupts are assigned individual enable bits which must be written logic one together with the Global Interrupt Enable bit in the Status Register in order to enable the interrupt.

Depending on the Program Counter value, interrupts may be automatically disabled when Boot Lock bits BLB02 or BLB12 are programmed. This feature improves software security.

When an interrupt occurs, the Global Interrupt Enable I-bit is cleared and all interrupts are disabled. The user software can write logic one to the I-bit to enable nested interrupts. All enabled interrupts can then interrupt the current interrupt routine. The I-bit is automatically set when a Return from Interrupt instruction – RETI – is executed. There are basically two types of interrupts. The first type is triggered by an event that sets the Interrupt Flag.

19.Interrupt Response Time

The interrupt execution response for all the enabled AVR interrupts is four clock cycles minimum. After four clock cycles the program vector address for the actual interrupt handling routine is executed. During this four clock cycle period, the Program Counter is pushed onto the Stack.

20.AVR Memories

Overview

This section describes the different memories in the ATmega328P. The AVR architecture has two main memory spaces, the Data Memory and the Program Memory space. In addition, the ATmega328P features an EEPROM Memory for data storage. All three memory spaces are linear and regular.

21.In-System Reprogrammable Flash Program Memory

The ATmega328P contains 4/8/16/32K bytes On-chip In-System Reprogrammable Flash memory for program storage. Since all AVR instructions are 16 or 32 bits wide, the Flash is organized as 16K x 16. For software security, The Flash memory has an endurance of at least 10,000 write/erase cycles.

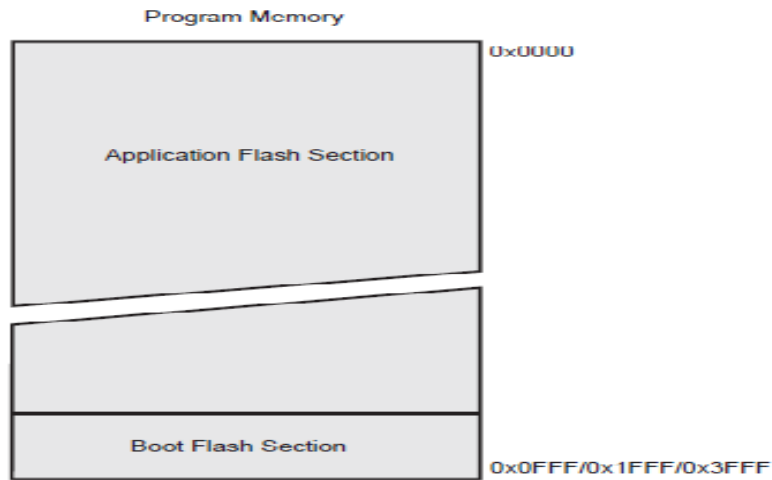


Figure 22. In-System Reprogrammable Flash Program Memory

22. SRAM (Static Random Access Memory) Data Memory

It shows how the ATmega328P SRAM Memory is organized. The ATmega328P is a complex microcontroller with more peripheral units than can be supported within the 64 locations reserved in the Opcode for the IN and OUT instructions. For the Extended I/O space from 0x60 - 0xFF in SRAM, only the ST/STS/STD and LD/LDS/LDD instructions can be used. The lower 768/1280/1280/2303 data memory locations address both the Register File, the I/O memory, Extended I/O memory, and the internal data SRAM. The first 32 locations address the Register File, the next 64 location the standard I/O memory, then 160 locations of Extended I/O memory, and the next 512/1024/1024/2048 locations address the internal data SRAM.

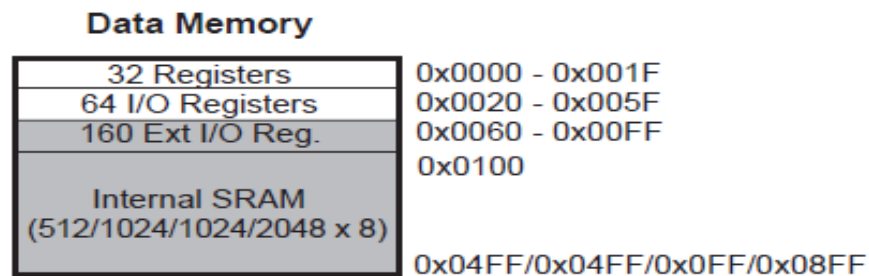


Figure 23. Data Memory Map

23.Data Memory Access Times

This section describes the general access timing concepts for internal memory access. The internal data SRAM access is performed in two clk_{CPU} cycles as described in the below figure.

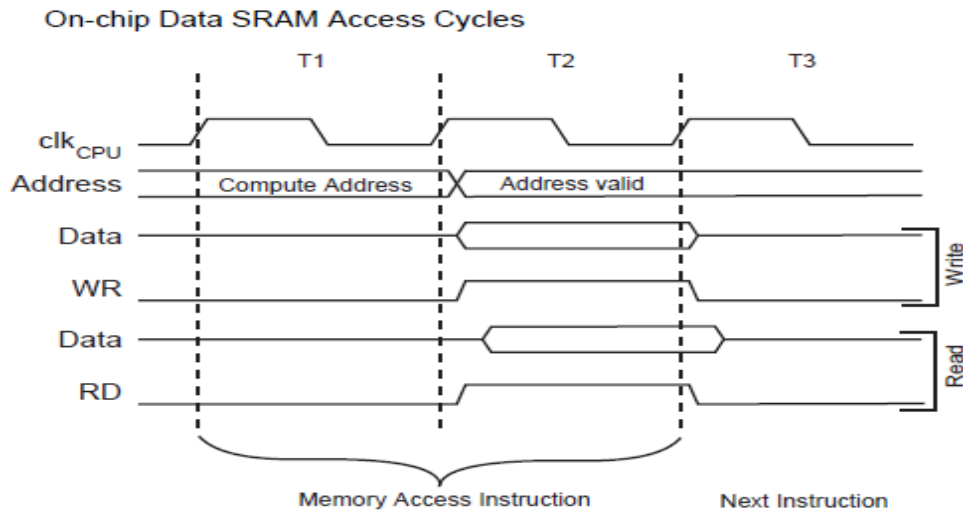


Figure 24. On- chip data SRAM Access Cycle

24. EEPROM Data Memory

The ATmega328P contains 1K bytes of data EEPROM memory. It is organized as a separate data space, in which single bytes can be read and written. The EEPROM has an endurance of at least 100,000 write/erase cycles. The access between the EEPROM and the CPU is described in the following, specifying the EEPROM Address Registers, the EEPROM Data Register, and the EEPROM Control Register.

a. EEPROM Read/Write Access

The EEPROM Access Registers are accessible in the I/O space. A self-timing function, however, lets the user software detect when the next byte can be written. If the user code contains instructions that write the EEPROM, some precautions must be taken. In heavily filtered power supplies, VCC is likely to rise or fall slowly on power-up/down.

b. Preventing EEPROM Corruption

During periods of low VCC, the EEPROM data can be corrupted because the supply voltage is too low for the CPU and the EEPROM to operate properly. These issues

are the same as for board level systems using EEPROM, and the same design solutions should be applied. An EEPROM data corruption can be caused by two situations when the voltage is too low. First, a regular write sequence to the EEPROM requires a minimum voltage to operate correctly. Secondly, the CPU itself can execute instructions incorrectly, if the supply voltage is too low. EEPROM data corruption can easily be avoided by following this design recommendation: Keep the AVR RESET active (low) during periods of insufficient power supply voltage.

c. I/O (Input and output)Memory

All ATmega328P I/Os and peripherals are placed in the I/O space. All I/O locations may be accessed by the LD/LDS/LDD and ST/STS/STD instructions, transferring data between the 32 general purpose working registers and the I/O space. I/O Registers within the address range 0x00 - 0x1F are directly bit-accessible using the SBI and CBI instructions. In these registers, the value of single bits can be checked by using the SBIS and SBIC instructions. Refer to the instruction set section for more details. When using the I/O specific commands IN and OUT, the I/O addresses 0x00 - 0x3F must be used. When addressing I/O Registers as data space using LD and ST instructions, 0x20 must be added to these addresses. The ATmega328P is a complex microcontroller with more peripheral units than can be supported within the 64 location reserved in Opcode for the IN and OUT instructions. For the Extended I/O space from 0x60 - 0xFF in SRAM, only the ST/STS/STD and LD/LDS/LDD instructions can be used. For compatibility with future devices, reserved bits should be written to zero if accessed. Reserved I/O memory addresses should never be written. Some of the Status Flags are cleared by writing a logical one to them. Note that, unlike most other AVRs, the CBI and SBI instructions will only operate on the specified bit, and can therefore be used on registers containing such Status Flags. The CBI and SBI instructions work with registers 0x00 to 0x1F only. The I/O and peripherals control registers are explained in later sections.

d. General Purpose I/O Registers

The ATmega48PA/88PA/168PA/328P contains three General Purpose I/O Registers. These registers can be used for storing any information, and they are particularly useful for storing global variables and Status Flags. General Purpose I/O Registers within the address range 0x00 - 0x1F are directly bit-accessible using the SBI, CBI, SBIS, and SBIC instructions (Robert, 2013)

B. Experimental Output

In order to find out the operation and effectiveness of the developed food sensor model various perishable food commodities was selected to detect the presence of microorganism at different testing time .

TABLE - 1
Developed Food Sensor to Detect the Micro organism in the Selected Food Commodities

Food Commodities	Sources	Testing Time									
		0		½ Hr		1 Hr		1 ½ Hr		2 Hr	
		G	T	G	T	G	T	G	T	G	T
Water	Tap water (Salt water)	82	24	93	26	104	27	115	28	149	28
	Drinking water (Fresh)	69	21	74	23	84	26	106	27	122	27
	Drinking water (Boiled)	72	26	84	27	91	27	103	28	110	28
	Packed Drinking water	79	22	82	23	97	25	84	26	102	28
Milk	Cow's milk from Vendor (Raw)	97	26	106	26	112	27	125	27	138	28
	Cow milk from Vendor (Boiled)	74	26	85	27	97	27	108	29	112	29
	Pasteurized milk (Raw)	81	20	92	24	105	27	118	27	137	28
	Pasteurized milk (Boiled)	73	26	84	26	92	27	101	27	125	28
Curd	Curd obtained from Vendor	69	26	82	26	102	27	115	28	124	28
	Curd obtained from Pasteurized milk	74	25	80	26	92	27	107	28	128	29
Cheese	Commercial Cheese	83	15	86	20	129	23	130	26	135	28
Butter	Commercial Butter	79	20	82	21	89	23	93	25	100	26
Fruit Juice	Sugar cane Juice	60	15	65	17	82	20	91	22	104	26
	Tomato Juice	69	20	77	21	88	23	92	25	107	26
	Mango Juice	83	22	102	25	126	26	93	27	112	27
Fleshy foods	Chicken (Boneless)	72	26	90	27	102	28	109	29	128	29
	Chicken (Liver)	69	25	85	27	101	28	115	28	127	28
	Mutton(Boneless)	76	25	87	27	90	27	105	28	126	28
	Mutton(Liver)	68	25	75	27	85	27	92	27	106	29

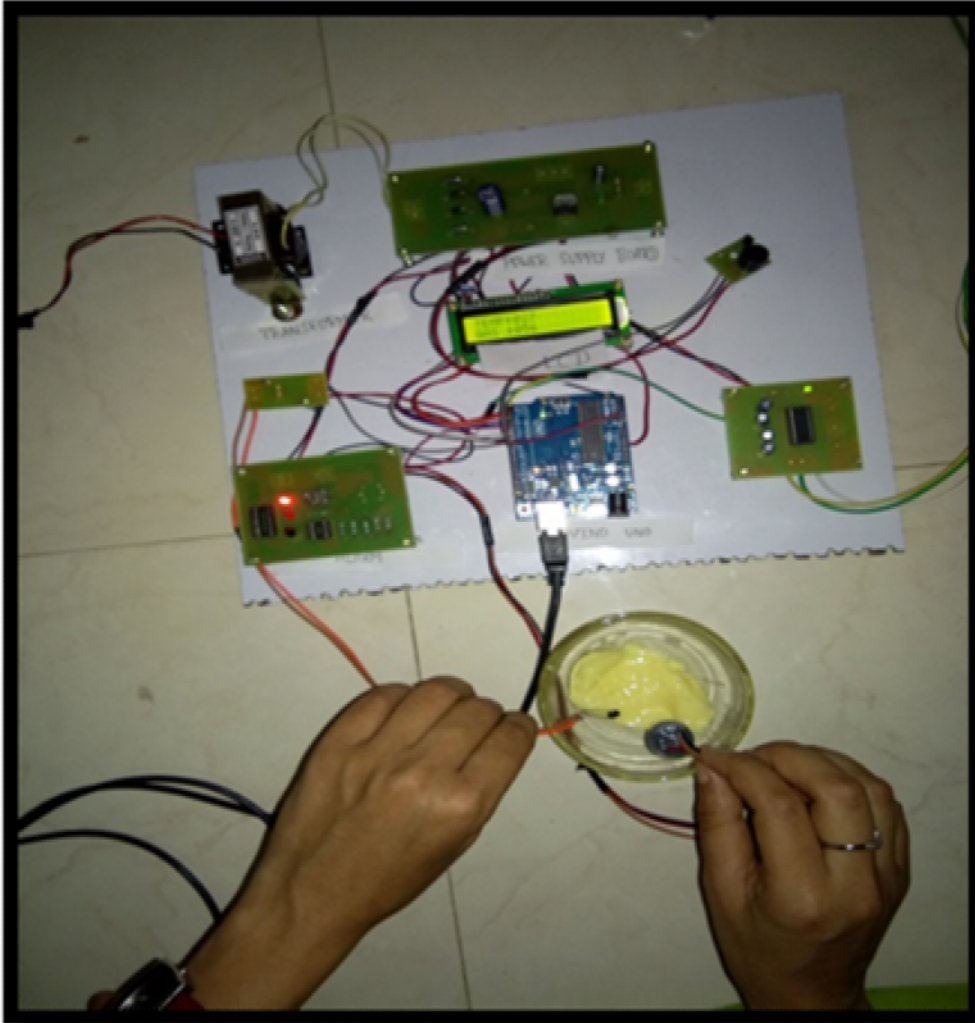
Food Commodities	Sources	Testing Time									
		0		½ Hr		1 Hr		1 ½ Hr		2 Hr	
		G	T	G	T	G	T	G	T	G	T
	Fish	78	26	89	28	108	28	122	28	142	29
Rice	Fermented rice	58	23	64	25	78	25	89	27	115	28
Idli Batter	Homemade idli batter	78	26	92	27	122	28	149	28	177	29
	Commercial idli batter	80	26	93	26	135	28	165	29	182	29

G-GAS

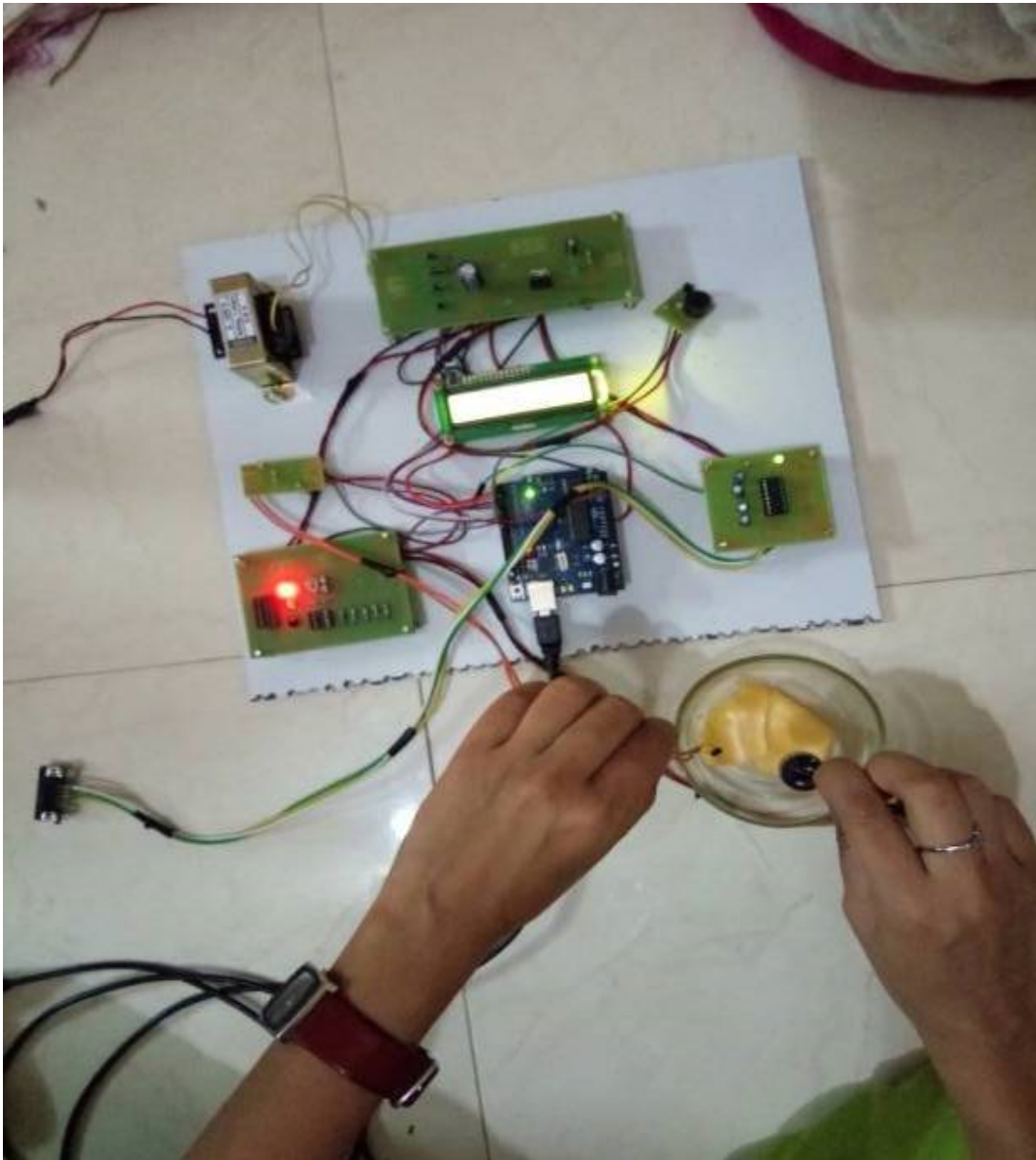
T-Temperature

PLATE 10. Output observation in different food commodities by using developed sensors.

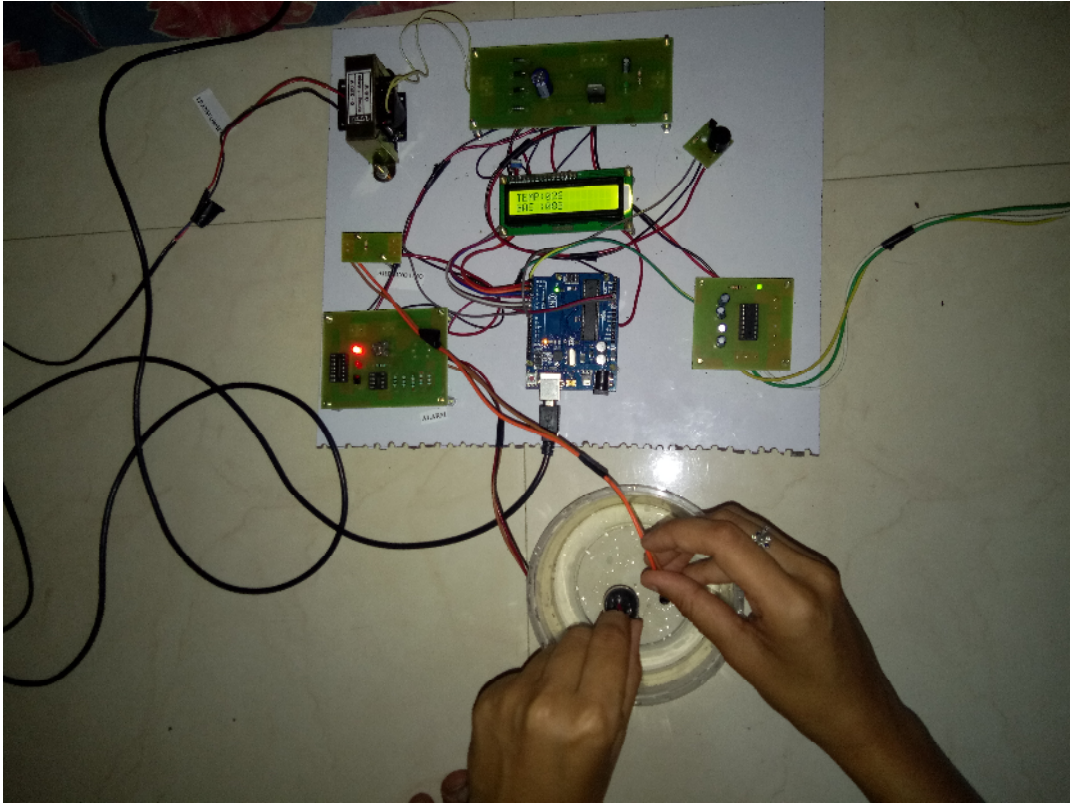
BUTTER



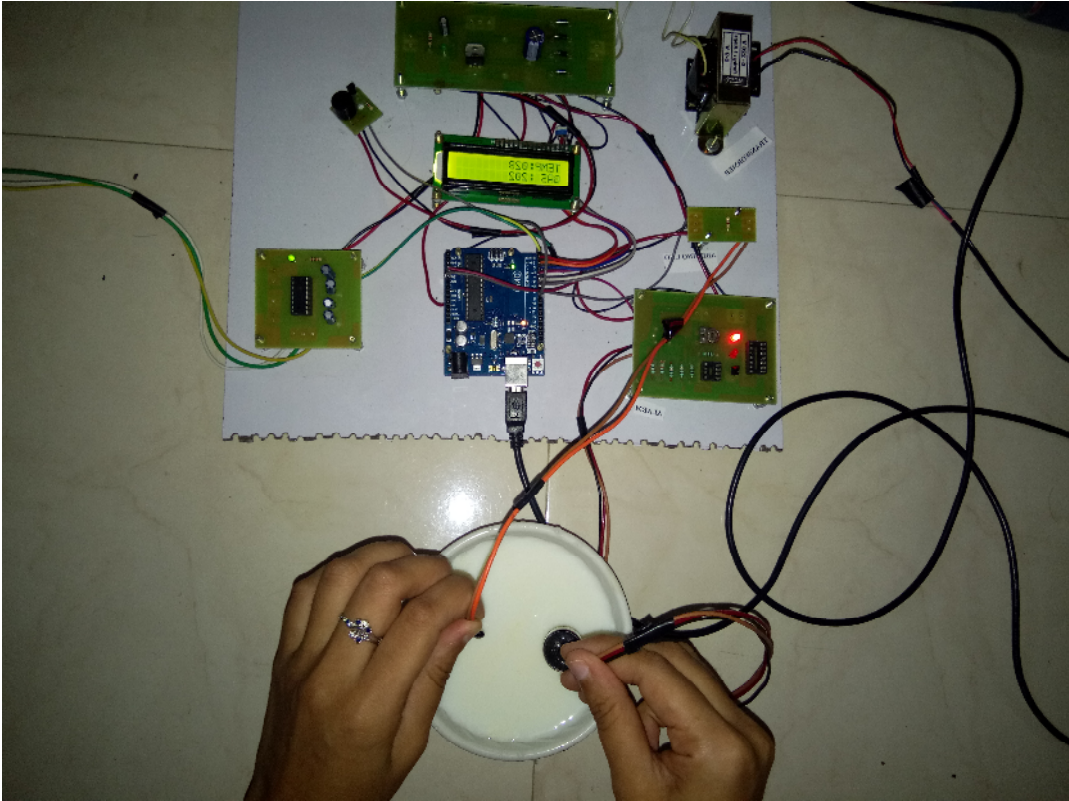
CHEESE



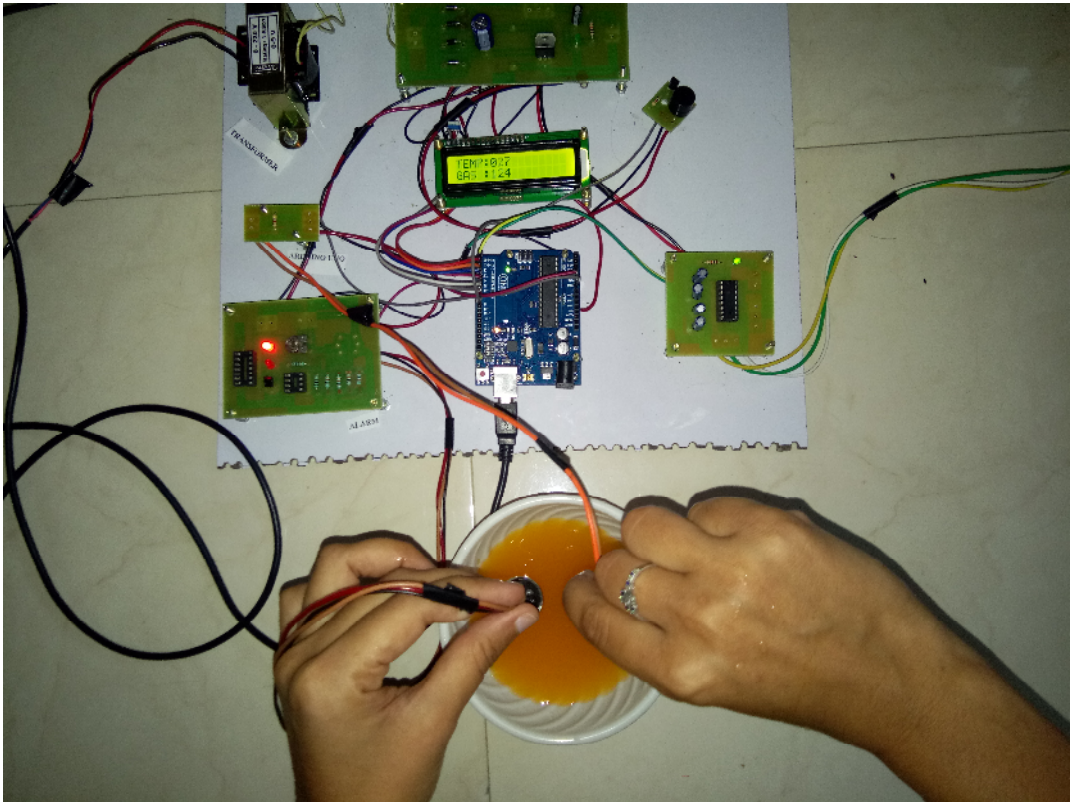
WATER



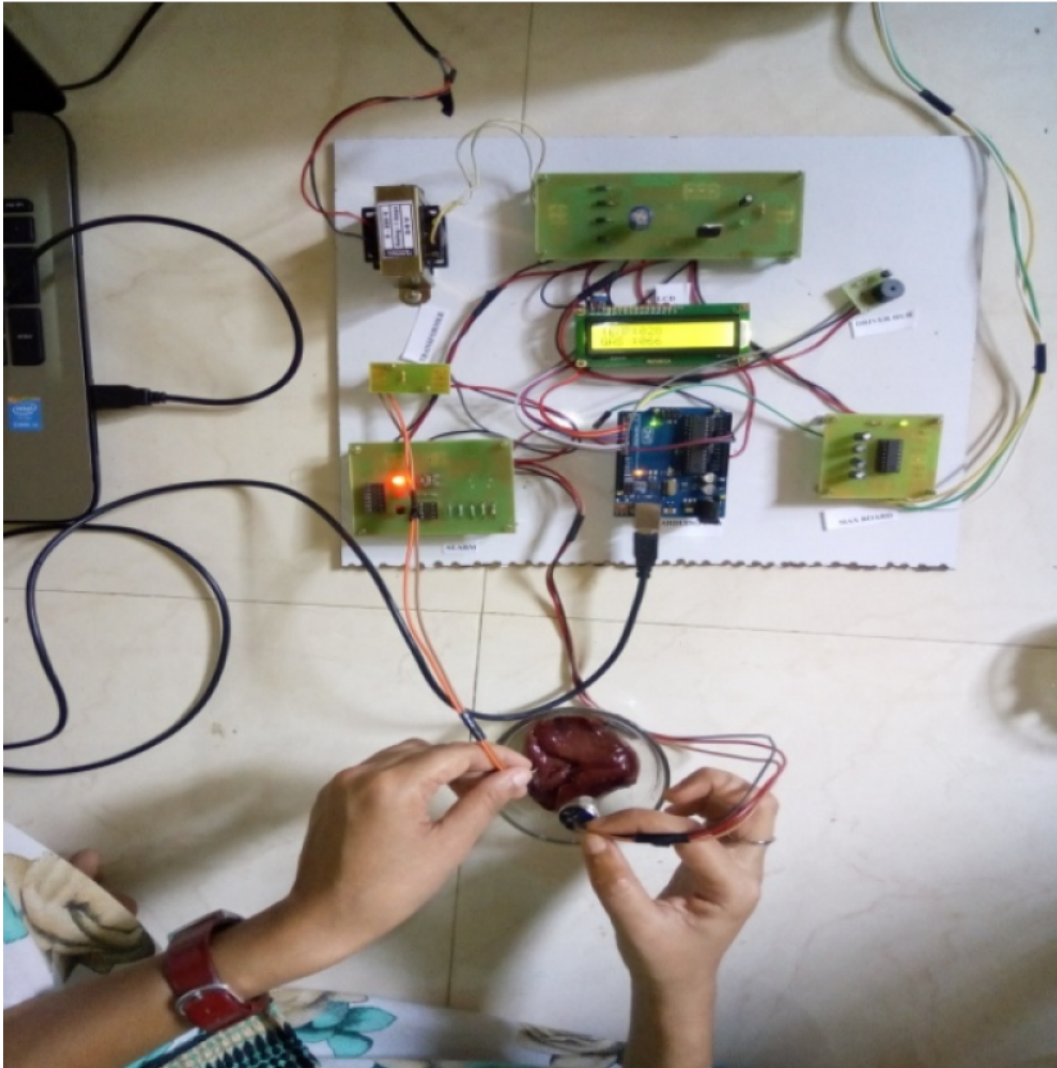
VENDOR & PASTURIEZED (BOILED AND BOILED MILKS)



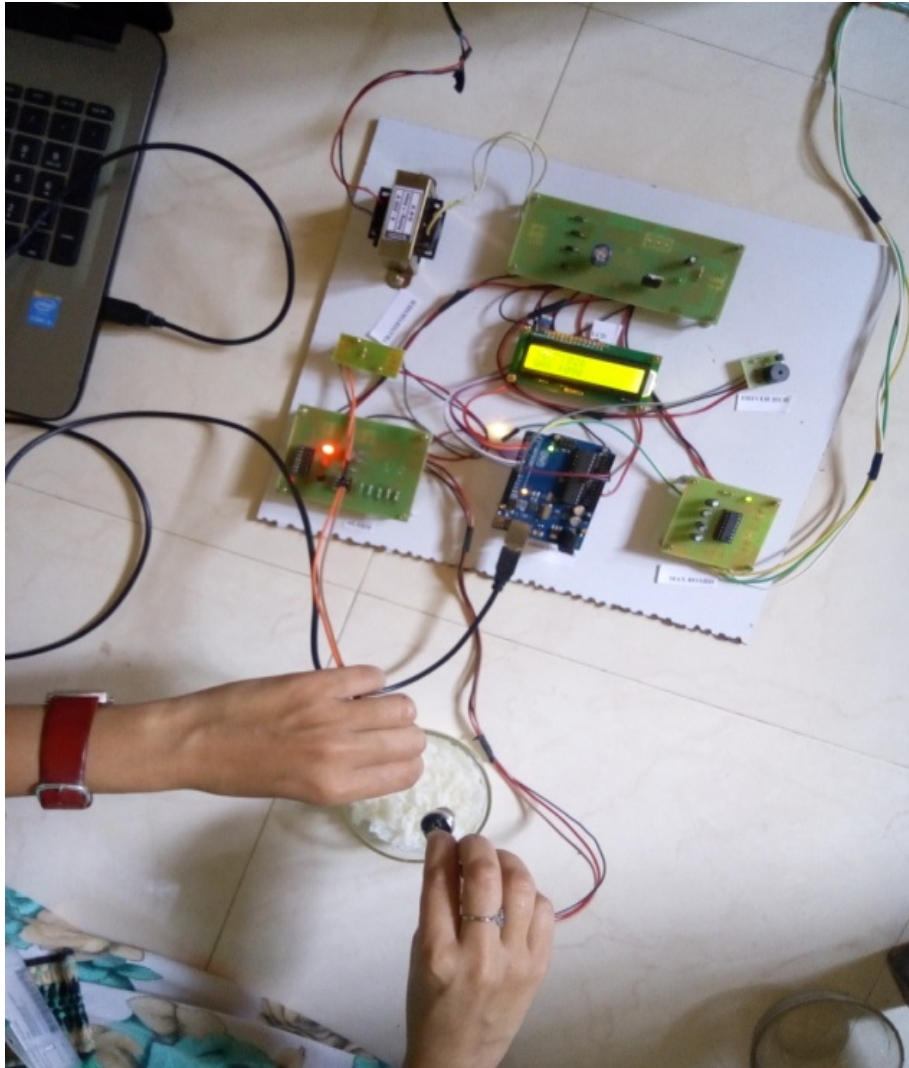
FRUIT JUICE



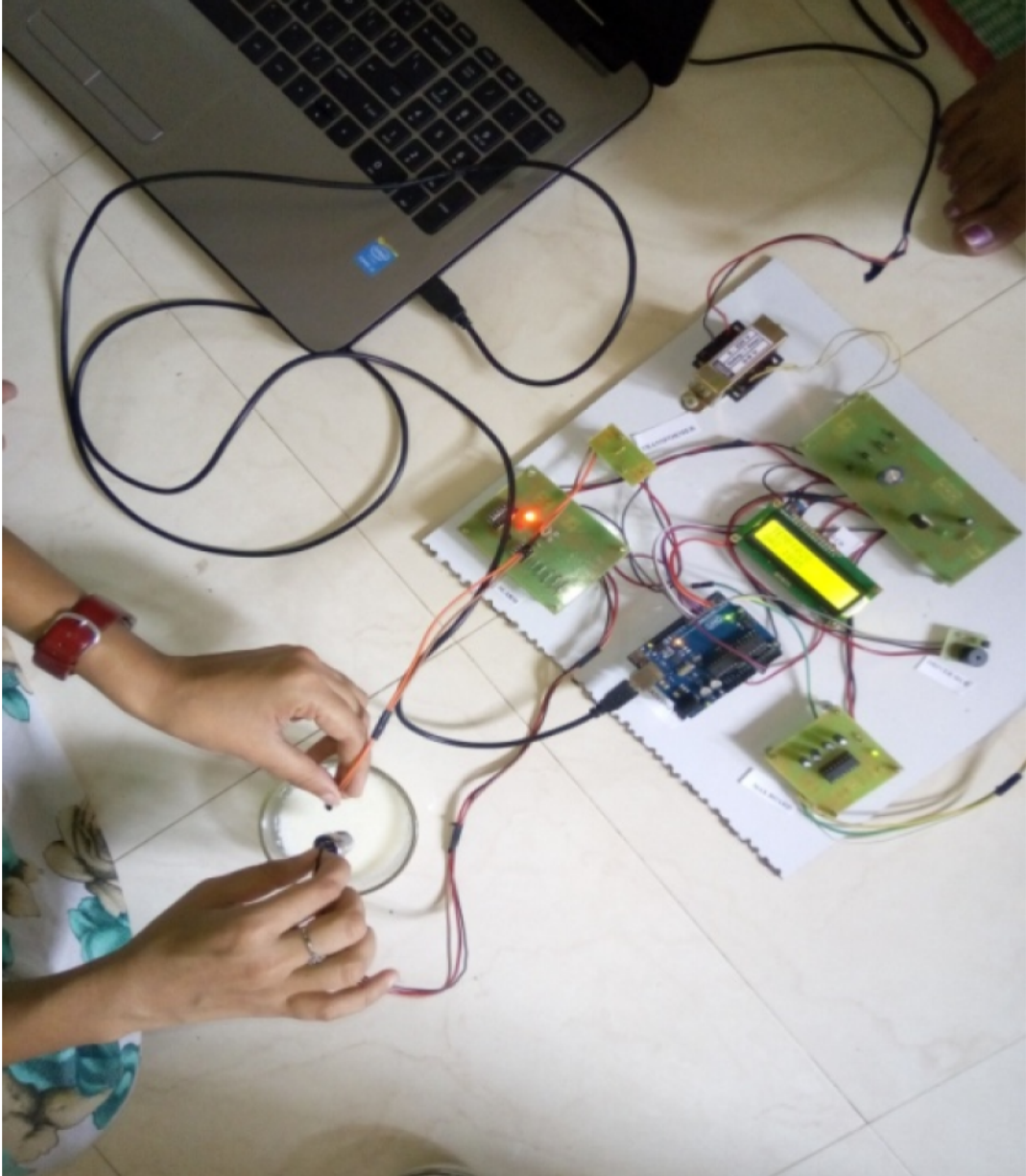
FLESHY FOODS



FERMENTED RICE & IDLI BATTER



CURD OBTAINED FROM VENDORS AND PASTURISED MILK



The results of the developed food sensor to detect the micro organism in the selected food commodities like water, milk, fruit juices, curd, butter, cheese, fleshy foods, fermented rice and idli batter was shown in above Table I.

Research results inferred that tap water got maximum production of gas after 2 hours reading in the LCD shows that 149ppm, 28°C when compared to other type of waters. Minimum production of gas observed in packaged drinking water 102 ppm at 28°C. At the time of 2 hours drinking water (Fresh) produced 122 ppm of gas with the respective temperature 27°C and drinking water (boiled) produced maximum 110 ppm of gas with 28°C temperature. Mainly water can be easily spoiled through microbial contamination like *campylobacter jejuni*, *Escherichia coli*, *Giardia Lamblia*, *salmonella* and so on are common pathogens contaminated and spoil the water.

When compared to milk obtained from different sources, raw cow's milk from vendors maximum production of gas at 2 hours the reading showed for the gas 138 ppm, temperature 28°C and cow's milk from vendors (boiled) produced minimum gas (112 ppm, 29°C). Pasteurized (raw milk) produced maximum 137ppm of gas with its respective temperature 28°C. Pasteurized (boiled milk) produced its maximum level 125ppm at the time of 2 hours at the temperature of 28°C. Milk can contaminate easily through *Bacillus cereus*, *Listeria Monocytogenes*, *Yersinia Enterocolitica*, *E coli* and *Campylobacter jejuni*.

Curd obtained from vendor milk got minimum production of gas (124ppm, 28°C) whereas in pasteurized milk got maximum production of gas (128ppm, 29°C).

Cheese got maximum production of gas on two hours (135ppm,28°C) *Lactococcus*, *Lactobacillus*, *streptococcus* are the bacteria contaminated in cheese which can be the responsible

Butter didn't got spoiled at time of 2 hours of observation due to the minimum of gas production (100 ppm, 26°C)

Among the three fruit juices, mango juice got maximum production of gas after 2 hours (112 ppm, 27°C) and In case of sugar cane juice produce minimum gas after 2 hours (104ppm,26°C). *E coli*, *Salmonella sp* and *Clostridium botulinum* contaminate the juices easily.

Fleshy foods can easily get spoil through contamination of pathogens like *Campylobacter jejuni*, *Salmonella Enteritidis*, *Stephylococcus Aureus* and *Listeria monocytogenes*. Fish got maximum spoilage after 2 hours (142 ppm, 29°C) and chicken liver got minimum production of gas after 2 hours (127ppm, 28°C). Incase of chicken (boneless) gas production was seen at 128ppm with the respective temperature of 29°C after observation of 2 hours.

After observation of 2 hours Mutton (bone less) produced its maximum level of gas was 126 ppm with 28°C of temperature. Mutton (Liver) produce 106ppm of gas after observation of 2 hours with the temperature of 29°C.

Idly batter procured from the commercial shop showed a maximum amount of gas after 2 hours observed value showed that 182ppm, 29°C and homemade idli batter get minimum production of gas when compared to commercial batter at 2 hours (177 ppm, 29°C).

The reference value of ethylene is 100ppm, if it is above 100ppm ethylene converted into ethane, ethane oxide through this reaction food will get spoiled easily (www.catalyticgenerators.com).

V. SUMMARY AND CONCLUSION

The term food sensor is defined as a device including control and processing electronics, to produce an output that is a measure of that quality. Biosensor is a biological detection system consists of a biological component combined with a transducer to perform measurement of a biochemical quantity. Biosensor is an analytical device which integrates the biological elements with the physiochemical transducer to produce an electronic signal is proportional to a single analyte and which is fetched into a detector

The term biosensor was developed in 1960s to define the use of bio electrodes involving enzyme electrodes that presented and bio catalytic activity. In 1962, Leyland C. Clark brought up the very rudimentary model of biosensors forward with the early invention of an oxygen electrode in which the electrochemical detection of oxygen or hydrogen peroxide could be used for a broad choice of bio analytical purposes.

Biosensors research is booming around the world and the area of applications range from medical to agriculture. The types of instruments required for the agro-food diagnostics market can be divided into large multi-analysers, bench-top portable instruments and one shot disposable sensors.

Biosensors are very useful in food borne pathogen detection. For instance, they have sensitivity in the range of mg/ml for microbial toxins; provide fast or real-time detection; and, the miniaturization of biosensors allow for integration in food production equipment and machinery.

USA remains as the most important country in terms of research in biosensors, followed by China and Germany, the period of last 5 years (2009-2014) China's influence in this field is gaining momentum and countries like South Korea and India are increasingly researching in these areas with a higher number. This trend will continue with biosensors becoming smaller through miniaturization into the nanoscale range, the number of applications and research devoted to this field will increase

Biosensors in food industry are used for mainly two purposes. First is enzyme biosensor, which is mainly used in liquor and beverages industry for detecting or measurement of carbohydrates from alcohol, amino acids, amines, amides, phenol etc.

Biosensors have more advantages to detect pathogens in foods rapid and continuous measurement high specificity, very less usage of reagents required for

calibration, fast response time and ability to measure non-polar molecules that cannot be estimated by other conventional device, time consuming and we can monitor the pathogen content and spoilage easily.

Biosensors are an important alternative in the food industry to ensure the quality and safety of the food products and process controls with effective, fast and economical methods. The use of enzymatic biosensor technology in the food processing, quality control and on-line processes is compared to conventional analytical technique

In the present study was conducted in seven phases. In first phase development of food sensor was carried out. For developing the sensor 220 V transformer was used, it step down 220V into 9 V as a AC V. Power supply board was also used to convert the AC V to DC V through bridge rectifier. Capacitor is used to filter the Dc V because current supply will be alternative. Regulator was also used to supply the accurate 5 V. Gas sensor was used to sense ethylene gas and temperature sensor was used to sense the respective temperature in common foods like water, milk, fruit juice, fermented rice, idli batter and fleshy foods.

The sensor have capability to sense the ethylene gas until 200Ppm and the temperature sensor have its capability to sense -20 to 50°C. Micro controller stores the input and it give the output through the signal. It transfers the signal to alarm zone and LCD display. Max board was used to send the signals and output to the system because PC will work only on 12 to 19V so these boards will convert the 5 V output and signals to 12 and19 V and send it to the PC. Liquid crystal display (LCD) display the output value and alarm will receives the signals and data from the microcontroller and it gives output through buzzer sound.

In phase II temperature and gas sensor will be placed downwards to the food sample. It will sense and monitor the gas and temperature values and send the value to the micro controller.

Incase of phase III milk, water, fruit juices, fermented rice, idli batter, butter, cheese, curds and fleshy foods are the food commodities were selected as sample because these foods are commonly used by everyone and also it can be contaminated by the pathogens very easily causes food borne diseases and food poisoning because of the production of ethylene gas, so these foods were selected.

In phase IV the developed sensor will sense the food and the value will be displayed in the serial monitor, the data displayed in the serial monitor will be read and compared with the reference value by the micro controller.

In phase V, if actual value was higher than the reference value, the sensor will give signal to alarm zone.

In phase VI if the real value is higher than the reference value, the sensor gives signals through microcontroller to the alarm zone. Through microcontroller, alarm zone receives the signal. When the researcher connects speaker with Arduino UNO while the alarm is ringing it gives the signal through the sound or the value will display through liquid crystal display (LCD).

In VII phase, two software are implemented for this study, Arduino UNO is used for coding and Lab view tools is a software used to run the output in the PC.

The results of the developed food sensor to detect the micro organism in the selected food commodities like water, milk, fruit juices, curd, butter, cheese, fleshy foods, fermented rice and idli batter was shown in above Table I.

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APPENDIX I

INSTITUTIONAL HUMAN ETHICS COMMITTEE



Avinashilingam

Institute for Home Science and Higher Education for Women

University

(Estd. u/s 3 of UGC Act 1956)

Chairman

Dr. S. Ramalingam
Principal, PSG Institute
of Medical Sciences
& Research, Coimbatore

Member Secretary

Dr. S.Uma Mageshwari
Associate Professor,
Department of Food Service
Management & Dietetics

Members

Dr. S. Kowsalya
Dr.P.R.Padma
Mr. K.Arulmoli (Legal Expert)
Dr. N.S. Rohini
Dr. A. Saraswathy
Mrs. V. Mangayarkarasi
Dr.Subhashini K. Sripathi
Mrs. S. Radha Devi
Dr.G.Victoria Naomi
Dr. Judith Justin
Dr.Anitha Subash

19th March 2018

To
Ms. Muthulakshmi.R
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Muthulakshmi,

Ref: Your proposal No. IHEC/17-18/FSMD/06 entitled
“Development of food sensor as an analytical tool in selected
foods” submitted for approval of the IHEC on 14th December.

The Institutional Human Ethics Committee of our University hereby
grants approval to your research proposal No.IHEC/17-18/FSMD/06
entitled “Development of food sensor as an analytical tool in
selected foods” submitted by you. The Approval number for the
same is AUW/ IHEC/ FSMD -17-18/XPD/06.

We wish you all the best in your research endeavours.

Regards,

S.Uma Mageshwari
Dr.S.Uma Mageshwari
Member Secretary



APPENDIX II

CODING

```
#include <LiquidCrystal.h>

LiquidCrystal lcd (7,6,5,4,3,2);

int alarm =8;

void LCD_Decimal3(unsigned int val);

void serial_Decimal3(unsigned char val);

unsigned int Temp, Gas, num, movee, k, Mic_count, server_time;

char m, val[10];

void setup()
{
  lcd.begin(16, 2);
  Serial.begin(9600);
  pinMode(A0, INPUT);
  pinMode(A1, INPUT);
  pinMode(alarm, OUTPUT);
  /* lcd.setCursor(0, 0);
  lcd.print(" WEARABLE ");
  lcd.setCursor(0, 1);
  lcd.print(" TEXTILE ");
  delay(4000);
  lcd.clear();
  */
}

void loop()
{
```

```

Temp=analogRead(A0)>>2;
Temp=Temp/5.5;

Gas=analogRead(A1)>>2;
  lcd.setCursor(0, 0);
  lcd.print("TEMP:");
  lcd.setCursor(5, 0);
  LCD_Decimal3(Temp);
  lcd.setCursor(0, 1);
  lcd.print("GAS :");
  lcd.setCursor(5, 1);
  LCD_Decimal3(Gas);delay(500);
  lcd.setCursor(8, 0);
  lcd.print("  ");
  lcd.setCursor(8, 1);
  lcd.print("  ");
  if(Gas>100 || Temp>28)
  {
    digitalWrite(alarm,HIGH);
  }
  else
  {
    digitalWrite(alarm,LOW);
  }
  Serial.print('*');
  serial_Decimal3(Temp);
  serial_Decimal3(Gas);

```

```

Serial.write(0x0d);
Serial.write(0x0a);
delay(2000);
}

void LCD_Decimal3(unsigned int val)
{
    unsigned char Lcd_h,Lcd_hr,Lcd_t,Lcd_o,Lcd_m;
    Lcd_m=val%1000;
    Lcd_h=Lcd_m/100;
    Lcd_hr=Lcd_m%100;
    Lcd_t=Lcd_hr/10;
    Lcd_o=Lcd_hr%10;
    lcd.write(Lcd_h+0x30);
    lcd.write(Lcd_t+0x30);
    lcd.write(Lcd_o+0x30);
}

void serial_Decimal3(unsigned char val)
{
    unsigned int Lcd_h,Lcd_hr,Lcd_t,Lcd_o;
    Lcd_h=val/100;
    Lcd_hr=val%100;
    Lcd_t=Lcd_hr/10;
    Lcd_o=Lcd_hr%10;
    Serial.write(Lcd_h+0x30);
    Serial.write(Lcd_t+0x30);
    Serial.write(Lcd_o+0x30);
}

```