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Urea Concentration Detection in Milk Using Microscopic Image Processing Algorithm under Ultraviolet Light Approach

Advay Bajaj
Strawberry Fields High School,
Chandigarh, India

Aaditya Sharma
Virginia Polytechnic Institute and State University,
Blacksburg, Virginia, United States of America

Abstract:- The adulteration of milk is a pressing concern for the citizens of India and people all around the globe. Due to a lack of regulation compliance and insufficient surveillance infrastructure, it is noticeably worse in emerging and slow-growing nations. One of the most common and dangerous adulterants in milk is urea. If the permissible quantity of urea in milk is surpassed, it could have a major negative impact on people's health. All existing methods of urea detection require time, expertise, costly chemicals, and enzymes, along with exorbitant instruments and instrument-specific expertise. The key to overcoming this challenge is having the infrastructure to detect adulterated milk. This study aims to identify a cost-effective and largely implementable system for quantitative detection of urea content to identify adulterated milk primarily for milk distribution centers in India. The proposed milk adulteration detection system, dubbed the MADS, entails a cost-effective, rapid, accurate, precise, and completely novel method for the quantitative computation of urea levels in adulterated milk. It is a device that detects the concentration of particles of urea in milk using a microscopic image processing algorithm under ultraviolet light. Using ultraviolet light and a proprietary program in Python, the isolation of the urea particle from the rest of the milk solids is done and the area concentration, as an average of the value calculated in each of the frames of the video captured through the microscopic camera, is computed. This gives the final urea concentration in milk, which can be used to check whether the concentration follows the government guidelines and exceeds the legal limit.

Keywords:- *Urea, Milk Adulteration, Image Processing, Quantitative Detection, Microscopic Analysis.*

I. INTRODUCTION

A. Problem Statement:

Due to its abundance of nutrients, which include amino acid sequences, lipids, carbohydrates (sugars and starches), and several beneficial organic compounds (at the macro level, including vitamins and minerals) that are necessary for both infants and adults, milk is referred to as the "ideal food" (Azad & Ahmed, 2016). India is the world's largest milk producer, accounting for 25% of worldwide milk supply with an estimated 210 million tonnes of milk produced every year. The nation's milk production has increased at an annualized compound growth rate of 6.2%. (*Page 277 - Economic_Survey_2021-2022*, n.d.). With 83 million metric tons of cow milk consumed yearly, India is the world's biggest milk consumer.

The adulteration of milk is a national issue for millions of Indians. Due to a lack of regulation compliance and insufficient surveillance infrastructure, it is noticeably worse in emerging and slow-growing nations. (Azad & Ahmed, 2016).

Urea, in addition to giving milk its characteristic white color, is added to milk to enhance its non-protein nitrogenous portion, balance proportions of SNF (solid-not-fat) in the milk, and increase its consistency. Additionally, urea serves as the main component in the preparation of synthetic milk. The health risks that urea poses include cancer, ulcers, indigestion, and acidity. The kidneys, liver, and heart are all harmed by urea. The kidneys are particularly susceptible since they have to function more intensively to remove urea from the human system. (Kandpal et al., 2012). The urea compound $[CO(NH_2)_2]$ has a molecular arrangement that can be considered a derivative of ammonia. Milk containing ammonia causes sensory abnormalities, regression, and loss of acquired speech (Reddy et al., 2017).

As a naturally occurring component of fresh milk, urea has a permitted limitation of 70 mg/100 ml stipulated in the Food Safety and Standards Authority of India (FSSAI) Act 2006 and the Prevention of Food Adulteration (PFA) Rules 1955. (0.07% concentration) (Hilding-Ohlsson et al., 2012) (Sharma et al. 2012). However, reports across the country have found substantially higher and potentially dangerous levels of urea concentration in milk. For this reason, having an appropriate technique and device for detecting tampered milk is essential.

This paper aims to identify a cost-effective, precise, accurate, and largely implementable system for quantitative detection of urea content to identify adulterated milk primarily for milk distribution centers in India.

B. Causes:

The motivation to adulterate milk with urea is largely economic. Protein content, freezing point, fat percentage, and SNF (solid-not-fat) percentage are frequently measured in order to evaluate the standard of milk. Adulterants are added

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to the milk in order to manipulate those parameters, falsely elevating the milk's purity. (Azad & Ahmed, 2016). For many people, adulteration is a simple means of generating income due to the sizable market for milk and milk products. It increases profit margins and lowers production expenses. (*DAIRY MILK & MILK PRODUCTS ADULTERATION, ITS TEST FOR IDENTIFICATION*, n.d.)

C. Effects:

If the regulatory limit for urea content in milk is exceeded, it may seriously harm human health and cause severe illnesses. Acidity, indigestion, ulcers, and cancers are linked health risks. Heart, liver, and kidney damage are all caused by urea. Due to their increased workload in eliminating urea from the body, kidneys are more vulnerable. Milk containing ammonia causes sensory abnormalities, mental retardation, and loss of acquired speech. Additionally harmful to the digestive system and intestines is urea found in milk. (Hilding-Ohlsson et al., 2012).

A survey found that 160 samples of milk from India had adulterants in them, the most prevalent of which was urea. Additionally, the study found that 4–38% of kids reported having headaches, 3–57% reported having visual problems, and 12.5–57.3% reported having diarrhea. The research found that urea was found to affect around 60–100% of the unprocessed milk specimens supplied to school eateries and 68.4% of milk samples from 1791 samples across 33 separate states. (Dutta et al., 2022)

While the primary motivation for milk adulteration is financial profitability, there are other factors at play as well, such as inadequate supply due to global population growth. Approximately 68% of milk that is given to customers does not comply with the guidelines (Reddy et al., 2017).

II. EXISTING METHODS OF UREA DETECTION

While quantitative detections of adulterants in milk are complex and varied, qualitative detections may be carried out with the aid of intricate chemical processes.

Table 1: A Detailed Overview of Each Industrially Practiced Method Prevalent in Milk Production Facilities

Method	Description	Disadvantages
Wet chemistry-based *para*-dimethylamino-benzaldehyde (DMAB) method.	This is the urea detection method recommended by the FSSAI (Food Safety and Standards Authority of India), with a threshold for identification of 0.2 grams per millilitre (0.02% urea), in which urea reacts with DMAB (in a mildly acidic solution) to produce a yellow hue at room temperature following milk protein precipitation. (Bector, 1998)	The chemical used in the process, dimethylamino-benzaldehyde, is quite an expensive chemical, Moreover, this is merely a method of qualitative detection, i.e., it only detects the presence of urea particles and cannot find the specific concentration levels or the amount of urea present in each amount of milk. It also has a very low detection limit and will not be able to detect urea in its high concentrations.
IR (Infrared) Spectroscopy	This method has been used to measure the amount of urea present without the need for any prior processing. (Khan et al. 2014).	It is time-consuming and requires instruments like an infrared spectrometer, a monochromator, as well as a Fourier transform instrument, all of which are costly, incapable of mass production, and require instrument-specific expertise. It is thus an unsuitable method for implementing at a large scale for urea concentration detection in the country.
GC/IDMS (Gas Chromatography/Isotope Dilution Mass Spectrometry)	Isotope dilution mass spectrometry is the process whereby natural isotope composition is measured by Mass Spectrometry and provides the concentration of the analyte in the sample after certain calculations. Gas chromatography is a method that includes inserting a gaseous or liquid sample into a mobile stage referred to as the carrier gas and conveying the gas through a stationary stage, can also be utilized as it allows for the separation of the compounds in the mixture. (Hall, 2023).	This is a complex process requiring costly chemical equipment, research laboratories, and laboratory professionals, rendering it impractical for mass testing of adulterated milk throughout the country.
HPLC (High-Performance Liquid Chromatography)	This technique has been shown to identify naturally occurring urea in milk, with the recommendation that the urea be changed into a derivative with a chromophore prior to HPLC analysis. (Patel & Patel, 2021)	HPLC is an expensive approach that involves a force supply, numerous expensive organics, and expert assistance. Additionally, it has limited effectivity for certain mixes, and some

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	.	are irreversibly adsorbed, making them impossible to distinguish (Timchenko, 2021).
A Combination of Kjeldahl and Spectrophotometric methods	This approach can assist in identifying milk adulteration caused by ammonium sulfate, urea, and melamine. The Kjeldahl method uses sulfuric acid digestion to convert nitrogen to ammonium sulfate, whereas spectrophotometry is a common and low-cost way of measuring light absorption or the amount of compounds in a solution.	Because various proteins have varied amino acid sequences and hence require different correction factors, this procedure is not scalable or feasible. Both the use of some potential catalysts and the high-temperature application of concentrated sulfuric acid present a significant risk. Applying this approach takes a lot of time as well.

➢ *The Solution*

The proposed solution- a Milk Adulteration Detection System, dubbed the MADS- entails a cost-effective, rapid, accurate, precise, and completely novel method for the quantitative detection of urea levels in adulterated milk.

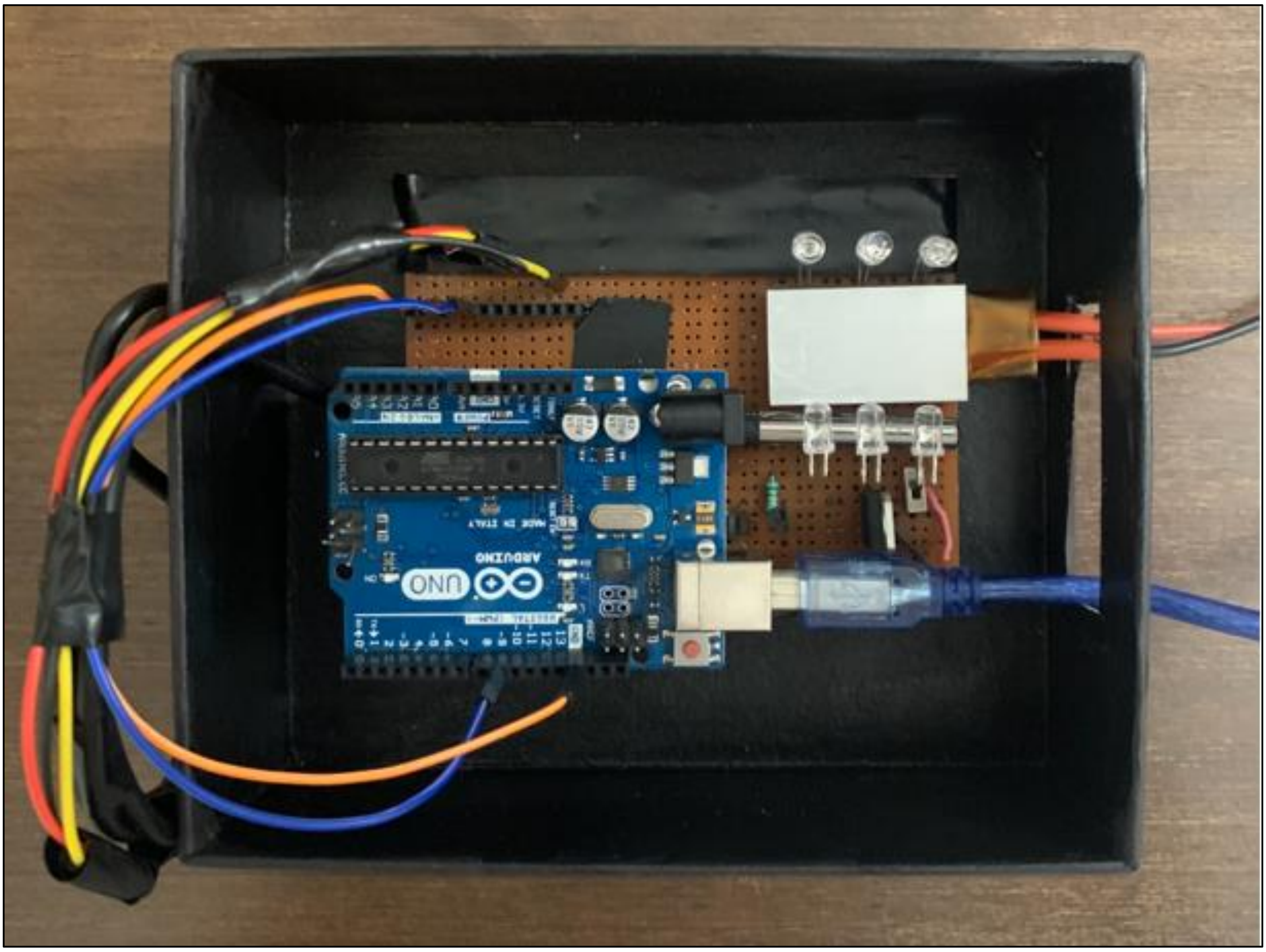

Fig 1: MADS - a Milk Adulteration Detection System
(Powered by an Arduino Uno controller, connected through wires to a Temperature Sensor, Heating Plate, UV LEDs, and a Power Source)

The user first turns on the device, with which the PTC heating plate activates and its temperature begins to rise. It continues to heat up gradually until the temperature sensor detects a temperature of 100.5 °C, the boiling point of milk, whereafter the temperature remains constant. The user will then place 4-5 drops (0.5 mL to 1 mL) of the milk sample onto the heating plate and simultaneously, the ultraviolet LEDs will switch on, casting their rays onto the surface of the milk.

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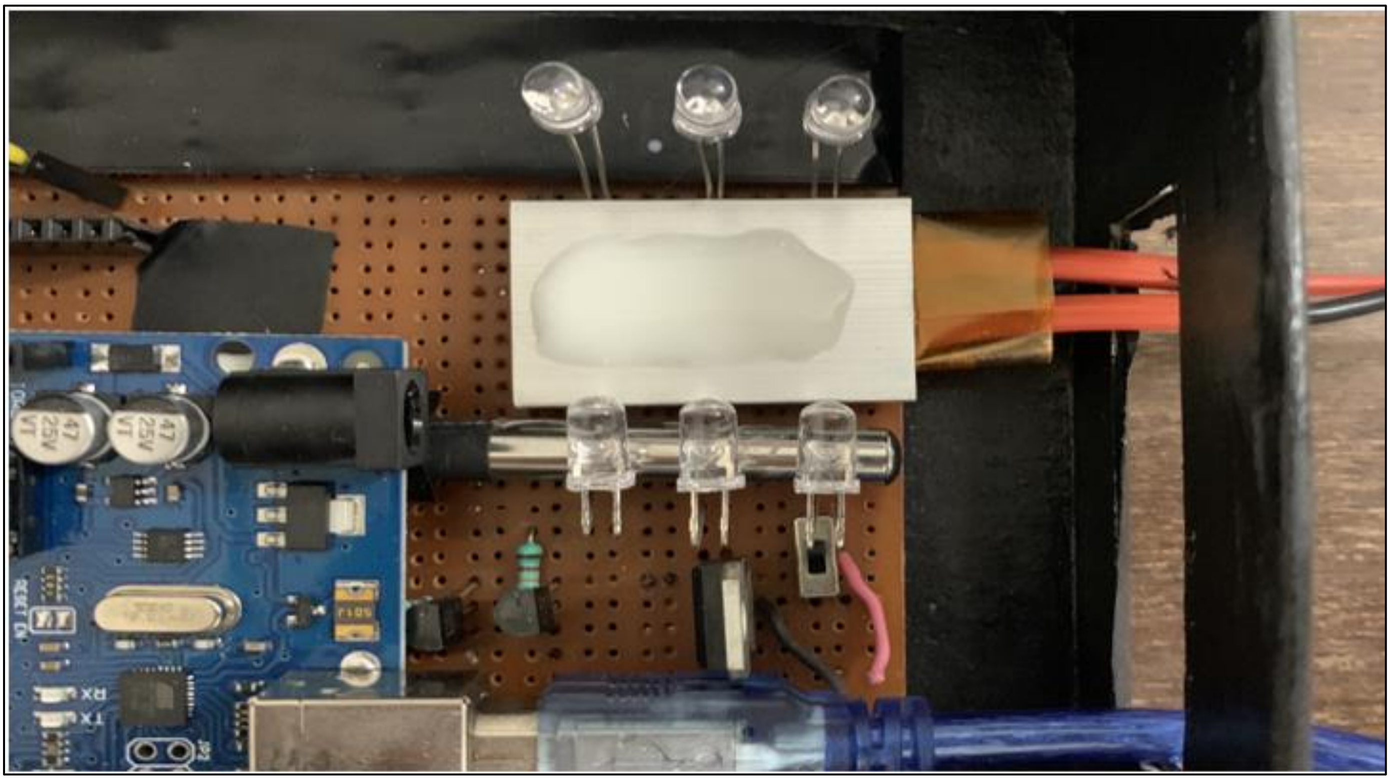

Fig 2: 0.5 mL to 1 mL Milk Placed on the Heating Plate

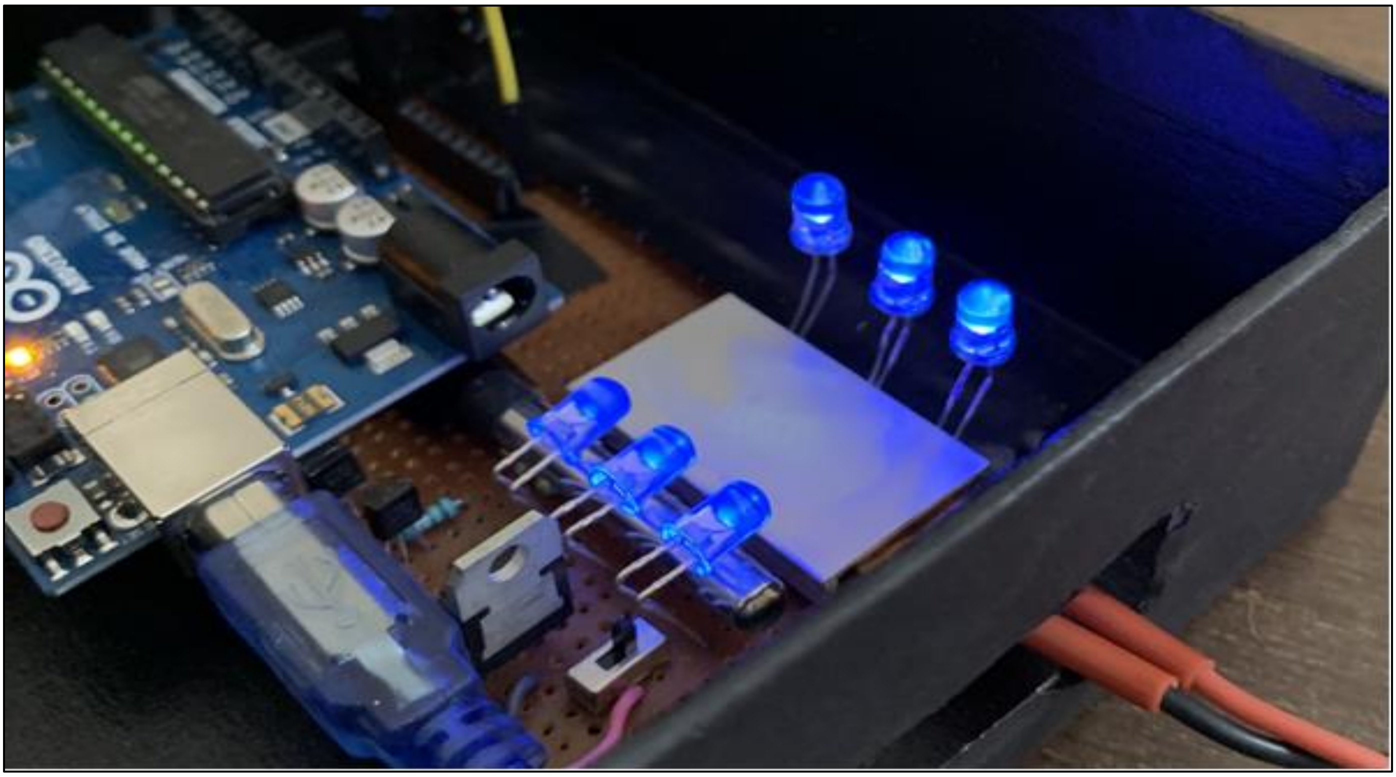

Fig 3: Ultraviolet LEDs Shining onto the Surface of the Heating Plate, Containing Evaporated Milk Mass

Subsequently, in about 10-15 seconds, the milk fluids (consisting of 87%-88% water) evaporate, leaving behind the milk solids. Along with these milk solids, urea particles will be present in the form of adulterants.

The user will then use the microscopic camera to observe the evaporated milk mass, which will record a video around 20 seconds long, getting more than 320 images (each scanning an area of 3mm x 2mm) for scanning (16 FPS). The urea particle is clearly visible as the whitish-translucent part that can be seen. This is because of the differential refractive indices of urea and milk solids, as well as the texture, smoothness, and nature of the surfaces of the particles which makes each type of particle reflect light in a different manner. As confirmed in an interview with a Ph.D. in Chemistry, this was correctly identified as a urea particle. All other colored particles are essentially milk solids, which consist of proteins (mainly amino acid sequences like lactalbumin and casein), carbohydrates (mostly disaccharides like lactose), and minerals (including phosphorus and calcium).

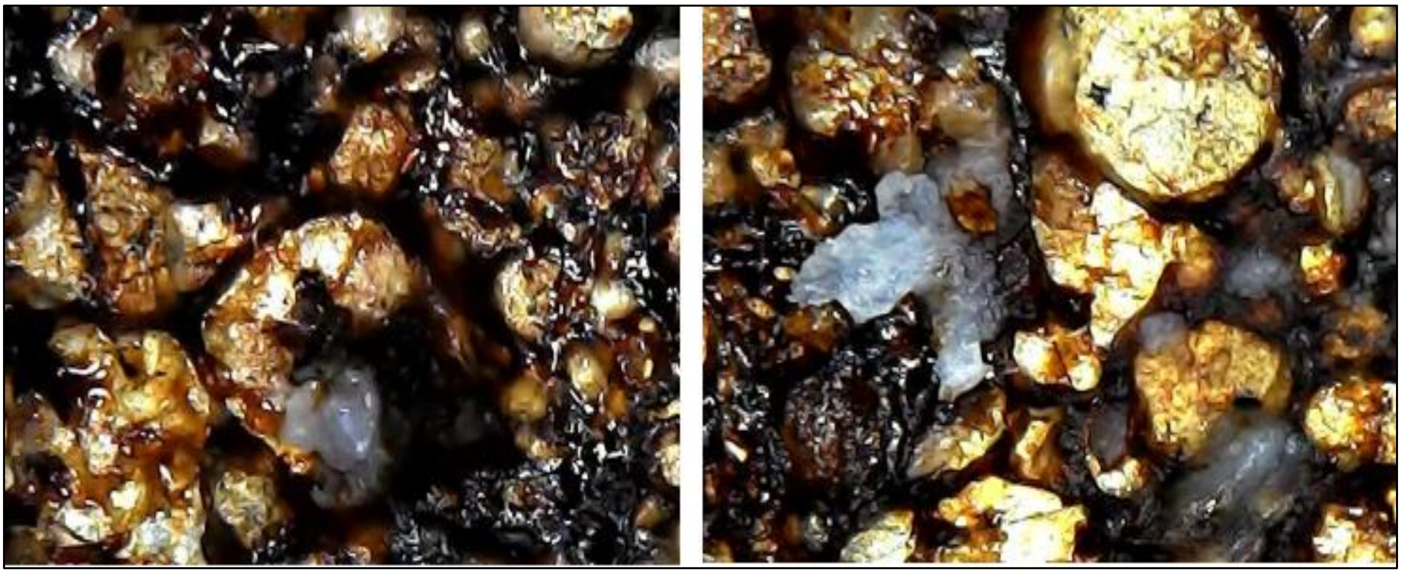

Fig 4: Microscopic view of burnt milk with artificially added urea for testing

The UV light helps isolate the urea particle in an even more pronounced manner since it becomes even more detectable and conspicuous.

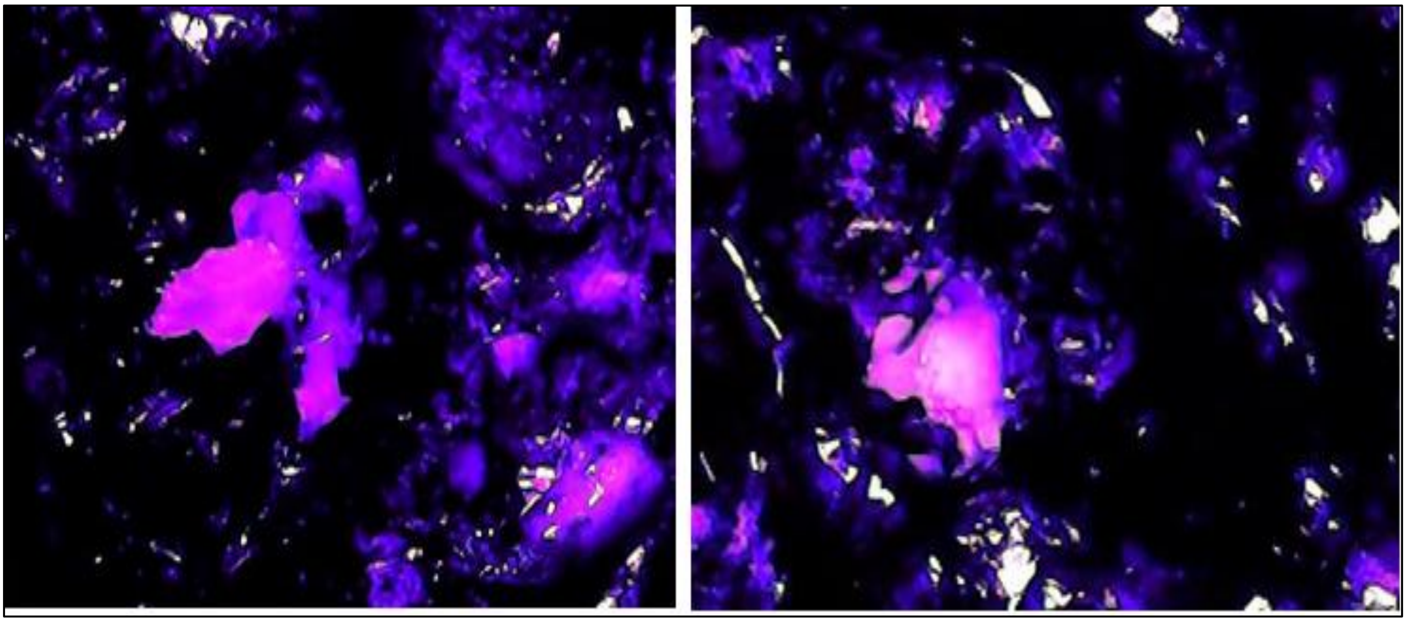

Fig 5: Microscopic view of burnt milk with artificially added urea under ultraviolet light

Then, using the code developed using the Python NumPy and OpenCV library, every frame of the video is effectively scanned through, helping to even further isolate the urea particle and make the urea identification indisputable and easily identifiable, based on HSV value modifications, i.e. by adjusting the HSV (hue, saturation, value) points of the image.

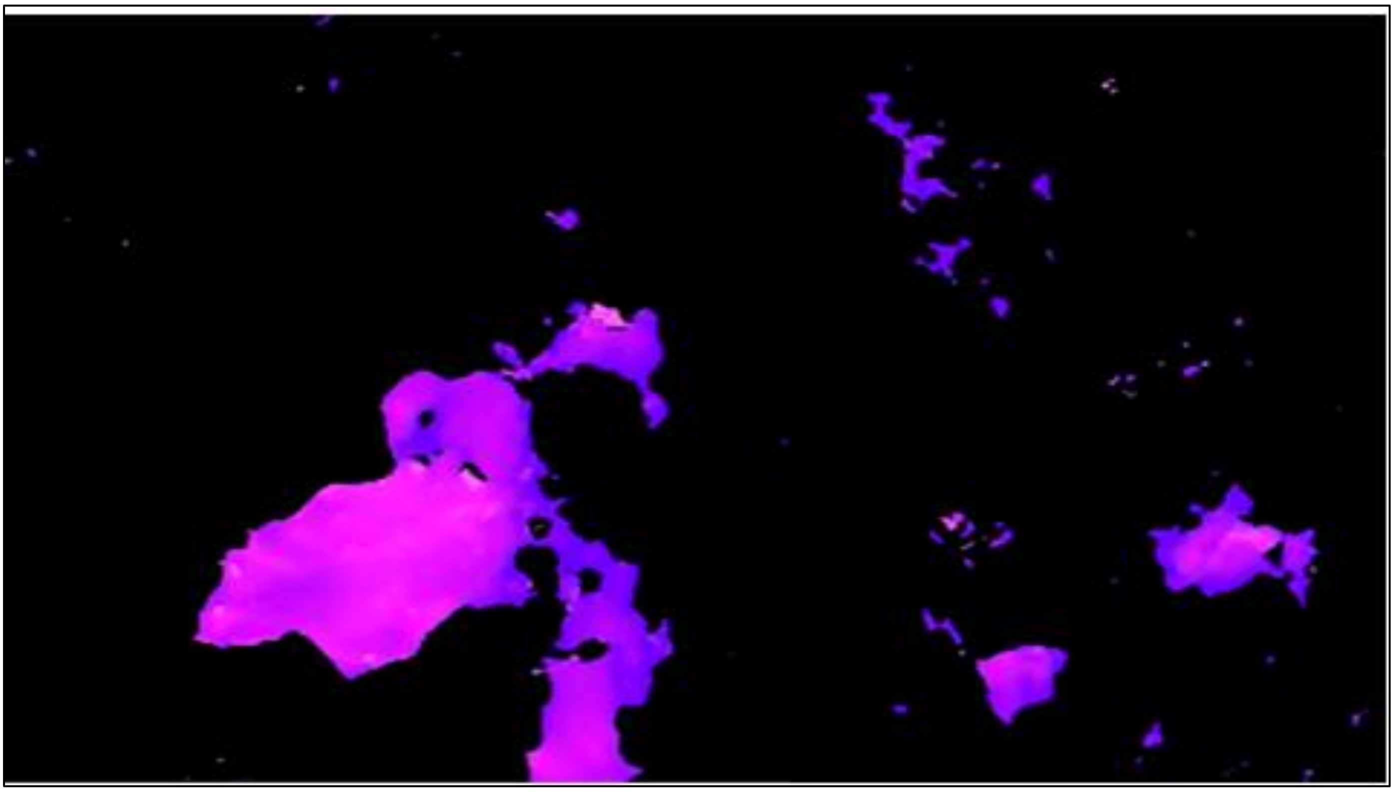

Fig 6: Microscopic image of isolated urea particle (via image processing algorithm)

The code then draws contours that go all around the urea particle and calculates the area of the particle, from which it can successfully calculate the urea concentration composition percentage.

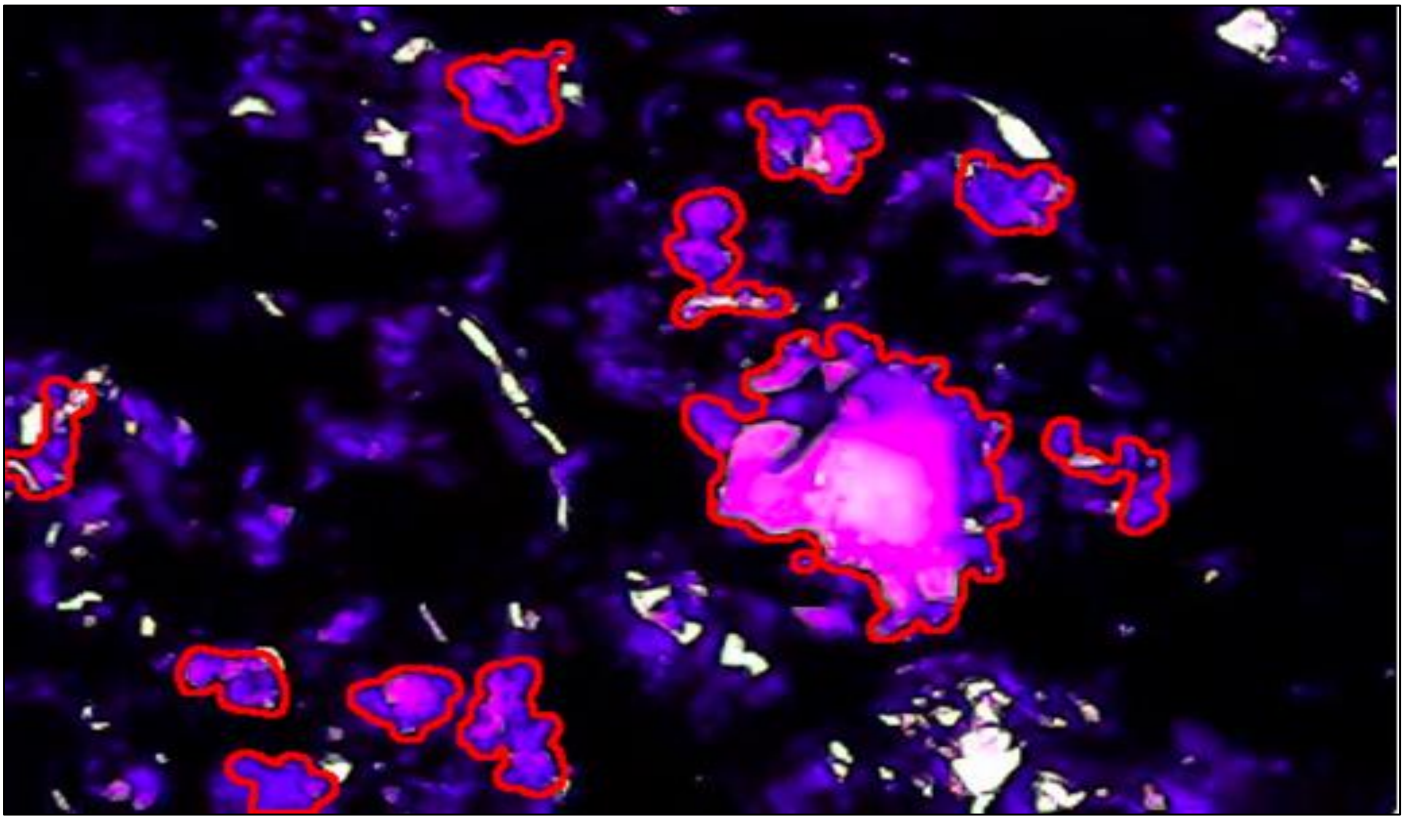

Fig 7: Microscopic Image of Urea Particles Surrounded by Red Contour Lines

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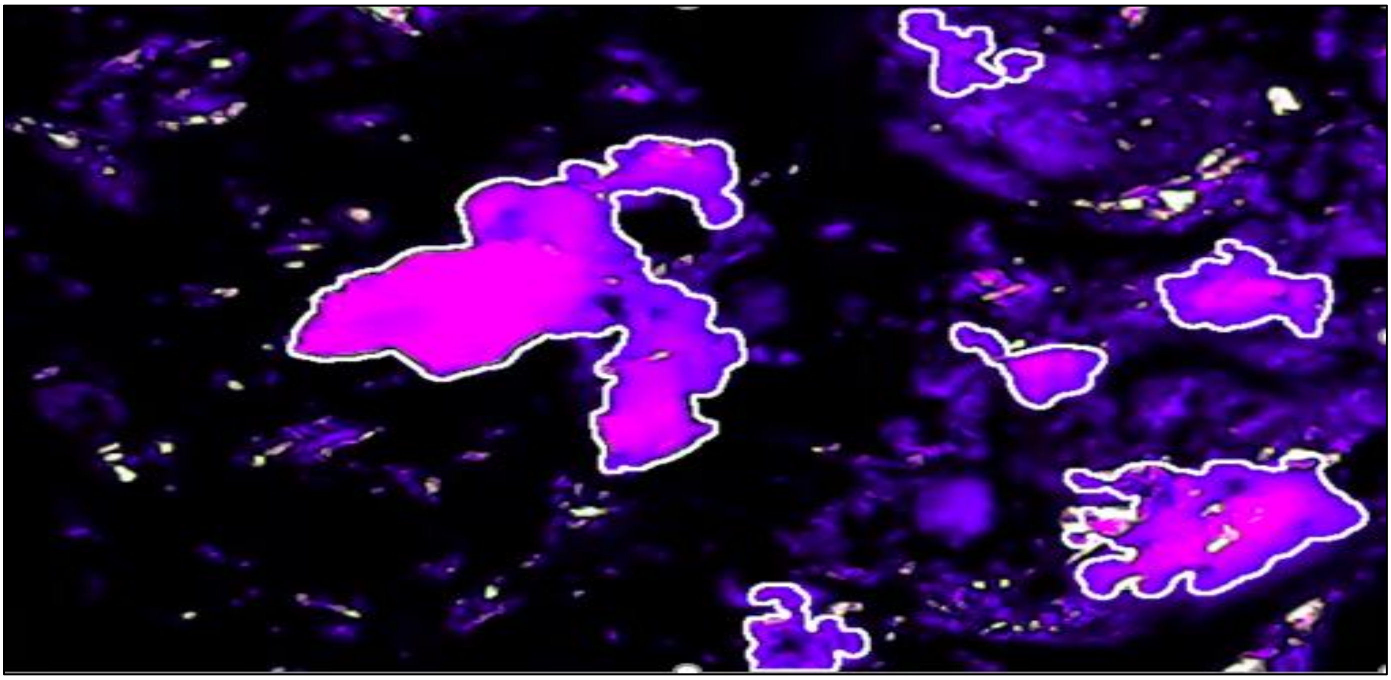

Fig 8: Microscopic Image of Urea Particles Surrounded by White Contour Lines

The code calculates the percentage of urea composition for every frame of the video recorded by the microscopic camera and, in the end, calculates the average percentage composition of all frames of the video. In this way, urea percentage composition in milk has been successfully calculated.

➢ *Within the Entire Code that has been Developed; Python Libraries have been used Which Include:*

- OpenCV- This helps in image processing, along with creating track-bars for image HSV modification and the entire contour drawing and isolating the urea particles
- NumPy- For mathematical operations and effective calculations
- sys- 'System' module which helps to use certain computer operation

III. RESULTS AND TESTING

Initially, the proposed system, MADS v1, simply took an image of the burnt milk residue and processed it through the devised algorithm. However, after conducting a series of tests using various milk control samples, it was found that the system, while precise, wasn't extremely accurate. The error rate was considerably high.

➢ *Test 1 - MADS v1*

- Urea - 0% (confirmed through arhar powder and litmus qualitative test) *
- Output - 0% urea concentration

➢ *Test 2 - MADS v1*

- Urea - 2% (artificially added for testing purposes)
- Output - 3.84% urea concentration

➢ *Test 3 - MADS v1*

- Urea - 5% (artificially added for testing purposes)
- Output - 5.67% urea concentration

➢ *Test 4 - MADS v1*

- Urea - 10% (artificially added for testing purposes)
- Output - 8.23% urea concentration

*First, the arhar powder test was used to assess the qualitative existence of urea in milk. For the experiment, combine one half of a teaspoon of soybean or arhar powder with a single teaspoon of milk in a test tube and shake thoroughly. Five minutes later, a red litmus paper is immersed in it. After half a minute, remove the paper. The occurrence of urea in milk is typically recognized by a color change from red to blue. However, the red color of the litmus paper remained unaltered following the test, indicating that there is no urea in the milk.

➢ *Test 5 - MADS v1*

- Urea - 20% (artificially added for testing purposes)
- Output - 16.93% urea concentration

Average Error - 1.47%

Through the conducted research, it was realized that when the milk was burnt, the urea wasn't spread evenly, and therefore simply processing an image independently wouldn't give an accurate method of quantitative detection.

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In the second version of the proposed system, MADS v2, instead of only processing an image, it was decided to shoot a video of the area of the burnt milk residue and process each frame of the video with the initial algorithm. After doing so, the values were averaged to get a more accurate result.

➢ *Test 1 - MADS v2*

- Urea - 0% (confirmed through arhar powder and litmus qualitative test)
- Video Duration - 30 seconds at 24 fps
- Total Number of Frames Processed - 720
- Output - 0% urea concentration

➢ *Test 2 - MADS v2*

- Urea - 2% (artificially added for testing purposes)
- Video Duration - 30 seconds at 24 fps
- Total Number of Frames Processed - 720
- Output - 2.01% urea concentration

➢ *Test 3 - MADS v2*

- Urea - 5% (artificially added for testing purposes)
- Video Duration - 30 seconds at 24 fps
- Total Number of Frames Processed - 720
- Output - 4.98% urea concentration

➢ *Test 4 - MADS v2*

- Urea - 10% (artificially added for testing purposes)
- Video Duration - 30 seconds at 24 fps
- Total Number of Frames Processed - 720
- Output - 10.02% urea concentration

➢ *Test 5 - MADS v2*

- Urea - 20% (artificially added for testing purposes)
- Video Duration - 30 seconds at 24 fps
- Total Number of Frames Processed - 720
- Output - 20.04% urea concentration

▪ Average Error - 0.018%

It is evident that in shifting to MADS v2, the error rate is near negligible. Accounting for manual error in artificially setting the urea content, it can be asserted that MADS v2 is nearly perfectly accurate.

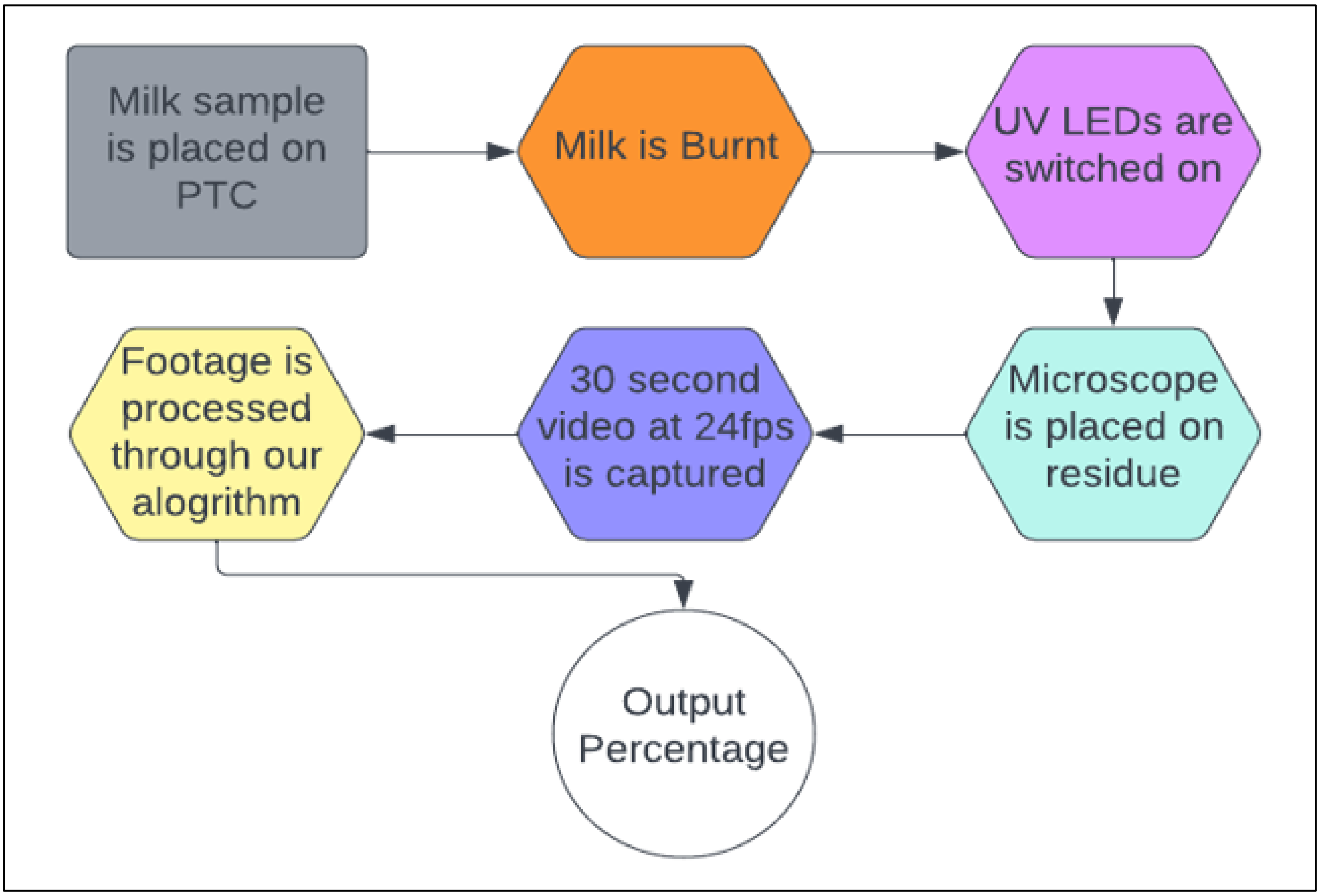

Fig 9: Process Flowchart of Urea Concentration Detection

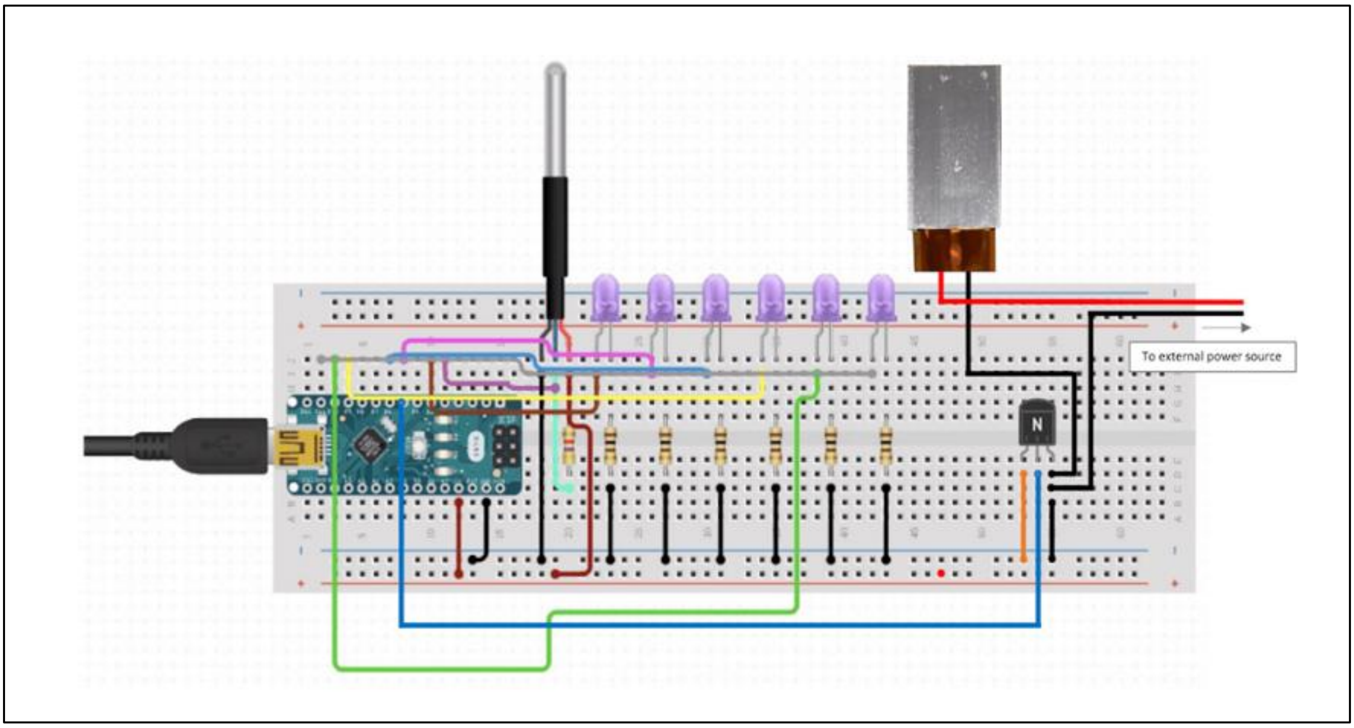

Fig 10: Circuit Diagram of MADS

Table 2: Components and Cost Breakdown

A Breakdown of the Components and Parts Used in Making MADS			
Component	Purpose	NOS	Cost
PTC Heating Element	Burning the milk	1	₹289
1000X Zoom USB Microscope with Camera Endoscope	Capturing milk residue footage for analysis	1	₹1,849
Ultraviolet LEDs	Isolate urea crystals from milk residue	6	₹36
DS18B20 Temperature sensor probe	Regulate PTC Temperature	1	₹247
12V 3A DC Power Supply Adapter	Power supply for PTC	1	₹375
Arduino NANO	Microcontroller for temperature sensor and UV LEDs	1	₹335
LM7812 IC - 12V Positive Voltage Regulator IC	Control switch for PTC	1	₹9
Total			**₹3,140**

IV. CONCLUSION

As it has been made evident through the abundant data, milk adulteration is an incredibly pressing crisis that India faces. Approximately 68% of milk provided to consumers does not meet regulations, and consuming urea through polluted milk can have negative impacts on human health.

The key to overcoming this challenge is having the infrastructure to detect adulterated milk. All existing methods of urea detection require time, expertise, costly chemicals, and enzymes, along with extortionate instruments and instrument-specific expertise.

MADS can reliably provide rapid, precise, and accurate quantitative measurements of urea levels in milk. Paired with its low cost of components and the consequent potential for scalability, it is believed it will play a key role in combating milk adulteration in India, specifically with urea.

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