Compare the Effectiveness of Natural and Commercial Food Preservatives on Food Samples Preserved using Simple MBRT and Spectrophotometric Analysis

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Abstract - *The purpose of our paper is to demonstrate the effectiveness of food preservation, using natural and commercial food preservatives, such that it retains its original nutritional values, colour and texture. Food preservation is a method to maintain food quality at an expected level so that we can get the maximum benefits. The deterioration and spoilage of food quality occurs mainly due to physical, chemical, enzymatic and microbial reactions. The major types of microorganisms that cause food spoilage and eventually food borne illness are bacteria and fungi. There are two categories of food preservation- i. the modern preservation method includes high hydrostatic pressure, high ionizing radiation, etc. and ii. the conventional preservation method includes drying, pickling, chilling, freezing and pasteurization.*

In our project, we have used both natural and commercial preservatives. We took honey, vinegar and salt as natural preservative, sodium benzoate and sodium nitrate as commercial preservative. As sample food products, we used orange juice, eggs, meat, milk and curd. We made separate solutions of each food product for control, natural preservative and commercial preservative. We preserved the food samples with specific amount of the preservatives for a particular duration. Eventually, we tested the preserved food samples using Methylene Blue Reduction Test, Spectrophotometeric Analysis and Durham's test tube to check the safer way of preserving food. Thus, the primary objective of our project is to impede deterioration of foods using preservatives (both natural as well as commercial) such that the shelf life of the food products increases and also the quality, appearance and taste is stabilized.

Key Words: **Food samples, Honey, Vinegar, Salt, Sodium Benzoate, Sodium nitrate, Methylene Blue; Spectrophotometer, Durham's tube**

1. INTRODUCTION

In this experiment, we have used natural and commercial preservatives for food safety purposes. We used honey, vinegar and salt as natural preservatives and sodium benzoate and sodium nitrate as commercial food preservatives. Honey is used as a preservative because of its antimicrobial properties. Honey contains various sugars and hydrogen peroxide. The bactericidal property and protective nature of honey can be used to preserve milk samples due to the presence of hydrogen peroxide [1]. Additionally, because of the high concentration of sugars in honey, bacteria and yeast cells cannot survive [2],[12]. Vinegar, an effective natural preservative, is produced by two types of fermentation; the first type is alcoholic fermentation [3] fand in the second type acetic acid is produced from *Acetobacter*. It is a liquid solution containing 5-10% acetic acid, pH (2.5) and vitamins and flavor compounds. It is mainly due to the pH or acidity of the vinegar which inhibits the growth of microorganisms and bacteria and this process is commonly known as pickling [4]. Most microorganisms that cause food destruction cannot survive in acetic acid environment. Moreover, the presence of certain bioactive components, such as acetic acid, gallic acid, and catechin in vinegar, are found to cause antioxidant, anti-diabetic and antimicrobial reactions [5]. Salt is the most well-known food preservative and flavoring agent. It helps to sterilize microorganisms through osmosis and thus maintains the food for longer period [6]. Low concentrations of salt stimulate microorganisms, but high concentrations inhibit them. A salt concentration of 20% kills bacteria. Sodium chloride releases moisture, creating a hazardous environment for bacterial growth. Salt reduces the water activity of foods. Excess salt is toxic to most microorganisms due to the osmolarity effect. Water circulates between the particles in the environment so that the concentration of salt on both sides of the cell is the

same. In most of the salt solutions, almost all the microorganisms break down due to the difference in pressure outside and inside the organism. Excess salt is also toxic to microbial internal processes that affect DNA and enzymes. Salt also incorporates yeast and molds [7],[11].

We used sodium benzoate and sodium nitrate as commercial food preservatives. Sodium benzoate is a common food preservative listed in the "Generally Safe" (GRAS) formulations by the United States Food and Drug Administration [8]. It is made by simultaneously synthesizing sodium hydroxide and benzoic acid meat [9]. When sodium benzoate is mixed with water, benzoic acid is produced, which is an active form of preservative that protects foods. It is also found naturally in some fruits such as apples and plums. It is used as bacteriostatic and fungistatic in acidic food and carbonated drinks, jams, fruit juices and spices. Sodium benzoate entering the food cells increases the overall acidity of the food and creates an environment where the fungus cannot grow and spread [10],[12]. Due to the high fructose corn syrup in carbonated drinks, it is found mainly in packaged drinks and beverages. In fact, it has been used as a preservative for many years due to its good consistency and excellent solubility in water.

When sodium nitrate is converted to sodium nitrite to be used as food preservative, it is sometimes added to the diet as nitrite storage. Nitrite is known for its antimicrobial effects against pathogenic bacteria. Nitrite also contributes to oxidative stress, a precursor of peroxynitrite ($ONOO^-$), which is the major strong oxidant. Sodium nitrate and sodium nitrite is often used to prevent spoilage from bacterial overgrowth in meat and fish [11]. In fact, they excrete moisture from bacterial cells and thereby protect food from spoilage [12].

2. MATERIALS AND METHODS

2.1 Samples and chemicals

Fresh fruit juice (Orange)-30mL, Egg (2-3), Processed meat (sausages)- 50-60 grams were collected from nearby available sources. A control sample of each food product was made without the addition of any kind of food preservatives. Natural food preservatives such as Honey (3-4mL), salt (20 grams) and vinegar (3-4 cups) were collected from variable sources along with Sodium Benzoate- E211(99.46%)- 10 grams was procured from Amazon.in. Sodium Nitrate (98.95%) - 10 grams was obtained from the chemistry laboratory of SBST department in Vellore Institute of Technology. Methylene blue (2-3 drops) was acquired from the laboratory for the first set of tests.

2.2 Apparatus and Instrumentation

Test tubes (5mL) and Durham's tubes were used to detect the presence of microbes based on the gas production as a result of their metabolism, for the second set of tests. For the third set of tests, we used spectrophotometer to determine the OD samples at various wavelengths (nm range).

Fig-1: Spectrophotometer

2.3 Mixed standard preparation

Sausage (processed meat) was blended using a blender and made into a thick paste for performing tests using methylene blue, spectrophotometer and Durham's tube.

2.4 Sample preparation

For the fruit juice, unpasteurized orange juice was pretreated with 1% honey(v/v) and 1% sodium benzoate (v/v). About 100% orange juice was taken as positive control. All the samples were stored on a shelf at $26 \pm 2°$ C for 24 hours, 5 days and 2 weeks.

For the egg, we made the control. The second sample by the method of pickling which is done using vinegar. For this we took a hard-boiled egg and mixed it with 4 cups of vinegar followed by refrigeration (in order to prevent the jar from breakage).

For the meat, we took about 50 grams of sausage, followed by cutting into small cubes and then covering it with layers of salt. For the commercial preservation, sodium nitrate salts were used instead of sodium chloride.

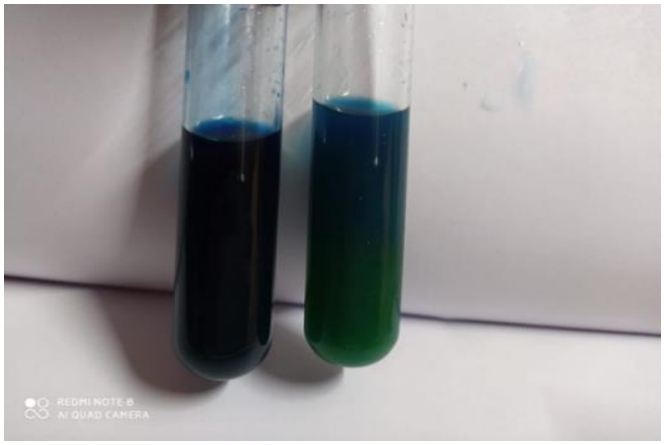

Fig-2: Methylene Blue Reduction Test of our food sample

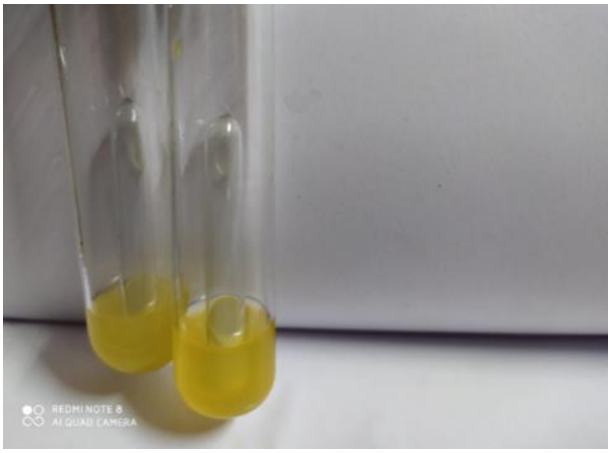

Fig-3: Durham's Tube Test of our food sample

Fig -4: One of our food samples

Fig -5: Some of the preparations for the experiment

Fig -6: Lab Preparations

3. RESULTS AND DISCUSSIONS

3.1 Methods and development

Three methods have been used by us to compare the effectiveness of natural and commercial food preservatives on some of the food samples prepared by us. The three methods used were Methylene Blue Reduction Test (MBRT), Durham's tube test and Spectrophotometric analysis.

We had prepared 3 food samples- control, food plus natural preservative and food plus commercial preservative. We followed the same methods for all the three food samples.

3.2 Methylene Blue Reduction Test (MBRT)

In MBRT we took 10 ml of all the 3 samples in 3 different test tubes. Then 2-3 drops of Methylene Blue was added to each test tube and was mixed well. After that the mouth of all the test tubes were sealed with aluminium foil. The observations for the MBRT test was taken within 8 hours of preparation, i.e. one at 0 h, next at 2 h, next at 4 h, then at 6 h, finally at 8 h. The following can be inferred based on the time taken for decolorization of the blue color of Methylene Blue-

Poor preservative-If decolorization occurs within 2 hours.

Fair preservative-If decolorization occurs within 6 hours but not less than 4 hours.

Good preservative-If decolorization occurs within 8 hours but not less than 6 hours.

Excellent preservative-If decolorization does not occur within 8 hours.

The following table shows the results we got for the MBRT test for the food samples prepared by us.

Table-1: Results of METHYLENE BLUE REDUCTION TEST

	Time (in hours)				
Samples	0	2	4	6	8
Control	Deep blue color	Rapid decolorization	Completely decolorized	Completely decolorized	Completely decolorized
Natural preservative	Deep blue color	No decolorization	No decolorization	Decolorization just started	Completely decolorized
Commercial preservative	Deep blue color	No decolorization	No decolorization	No decolorization	No decolorization

3.3 Durham's Tube Test

Durham's tubes are mainly used in microbiology for the detection of gas production by microorganisms. These tubes are placed upside down in bigger test tubes. The three food sample solutions were taken in three different test tubes. Then the Durham's tube was placed inside the test tube. Therefore, the Durham's tube also gets filled with the solution. The observations were taken based on the amount of air bubbles formed inside the Durham's tube. The Durham's tube which had the maximum amount of air bubble had the solution which developed maximum number of microorganisms, i.e. the control. The Durham's tube with a little amount of air bubbles contained the solution with a little amount of microorganisms, i.e. the sample with natural preservative. Finally, the Durham's tube with the least amount of air bubbles contained the least amount of microorganisms, i.e. the sample with commercial preservative.

The following table gives the details of the results we got for the Durham's tube test.

Table-2: Results of DURHAM'S TUBE TEST

	Time (in hours)		
Samples	24 hours	5 days	2 weeks
Control	Bubbles formed	Bubbles formed	Bubbles formed
Natural preservative	No bubble formation	No bubble formation	Little bubble formation
Commercial preservative	No bubble formation	No bubble formation	No bubble formation

3.4 Spectrophotometric analysis

The OD/absorbance test was carried out using a spectrophotometer under suitable wavelengths for the different food samples. The OD increases if the no. Of microorganisms in a solution, increases and it decreases if the no. of microorganisms decreases. Therefore the OD was found to be highest for the control, intermediate for the sample with natural preservatives and the lowest for the sample with commercial preservative. Control at 24 hours showed absorbance of 2.4220, sample with natural preservative showed 1.9448 at 24 hours and sample with commercial preservative showed absorbance 1.6582. These values showed rapid increase for the control but the increase in OD for the rest was not so rapid. The following table shows the comparison between the OD values for the different types of food samples.

Table-3: Results of SPECTROPHOTOMETRIC ANALYSIS

	Time		
Samples	24 hours	5 days	2 weeks
Control	High	High	High
Natural preservative	Low	Low	Low
Commercial preservative	Lowest	Lowest	Lowest

On observing all three samples under the microscope, maximum microbial growth was found in the control followed by sample with natural preservative and very less or nil in the sample with commercial preservative.

4. CONCLUSION

The proposed Methylene blue reduction test (MBRT), Durham's tube test, and spectrophotometry for simultaneous assay of natural and commercial preservatives such as honey, vinegar, salt, sodium nitrate, and sodium benzoate in food samples is simple and specific and therefore can be used to determine the

effectiveness of the prescribed preservatives. Among them, commercial food preservatives such as Sodium nitrate and Sodium benzoate are the most effective method to decrease microbial action followed by natural preservatives. Therefore, the methods and materials used are suitable for quality control of food samples.

5. DISCUSSION

The objective of the experiments was to compare the effectiveness of various types of preservatives, i.e. natural and commercial, on food samples and their effect on microbial action. Food samples along with the stipulated ratio of preservatives were observed at regular time periods. Finally, a mixture of food samples and commercial food preservatives proved to be more effective when compared to a mixture of food samples and natural preservatives.

In the case of the Methylene blue reduction test (MBRT), the basic objective is to determine the quality of food samples used, by observing the color change that occurs in the sample after the dye is added into it. Due to the formation of reducing substances, bacterial metabolism causes a decrease in oxygen in the food sample. Since methylene blue is an indicator of redox reactions, we can identify if the food preservative added to the sample is effective enough to control the bacterial growth and thereby prevent spoilage. When the dye is exposed to a sample that lacks oxygen, it loses its color. The time taken by the dye to get reduced and lose its color gives us an idea about the number of microbes in the food sample and the effectiveness of the food preservatives. From the results, we observe that food samples without added preservatives i.e. control reduces the dye within 2 hours of its addition. This shows the active metabolism of bacteria that is taking place and that the food is getting spoilt. Whereas the food samples with added natural preservative didn't show any color change for the first few hours, but within 6 hours, it slightly started turning bluish-green. The samples that contained commercial preservatives (sodium benzoate or sodium nitrate) did not show any visible color change within 8 hours. On observing all three samples under the microscope, maximum microbial growth was found in the control followed by sample with natural preservative and very less or nil in the sample with commercial preservative.

In the case of Durham's tube test, we are checking for the formation of bubbles. This is because microorganisms ferment food samples which results in the formation of an acid or an acid along with gas. Depending upon the amount of metabolic activity taking place in the food samples, the results vary. So, we observe that the control with Durham tubes showed the formation of bubbles at the earliest while the ones with added preservatives had the least or no bubble formation even after storing for a long period of time.

Spectrophotometric analysis is one of the methods of choice for measurements of the growth of bacteria. The basic abstraction behind the absorbance test is to quantify the turbidity of broth using a spectrophotometer based on which the number of bacteria in the broth can be anticipated and it is known as turbidometry [7]. This method is based on Beer and Lambert's law. Optical Density (OD) is directly proportional to the biomass in the cell suspension specific to the cell type in a given range, which implies that the food sample with effective preservative has the lowest OD as the bacterial metabolism is taking place least in it. On the other hand, after a certain period of time, bacterial growth increases rapidly in the control which gives a shift in the OD levels, increasing it to a higher peak. The limitation of this method is its inability to give an absolute count or distinguish between living and dead microorganisms. So, all the samples were observed under the microscope to get the exact idea of the microorganisms.

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