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Study of the Combinatorial Effect of Two Chemical Preservatives and A Natural Preservative on Microbial Spoilage of Avocado (*Persea americana*)

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Abstract

Persea americana is a seasonal fruit available only for a short period of time with a small shelf life period of three to six days. Its shelf life can be increased upon refrigeration up to few more days. However, the best way to preserve any comestible is by the use of preservatives after slicing it. This method ensures retaining the quality, chemical composition, texture and colour of the fruits. In this research work, Avocado slices were stored at room temperature to study the combinatorial effect of two chemical preservatives (Sodium Benzoate and Potassium Metabisulphite). Additionally, Avocado slices were stored in *Aloe vera* gel which acted as a natural preservative. The chemical preservatives were used in a fixed ratio with increasing degree of concentration to check which is the most effective in preserving the fruits. The physiochemical properties (pH, Acidity, reducing sugar content, and protein estimation) and Microbial growth (bacterial and fungal) of the pulp were studied for 40 days after the regular interval of 10 days. It was observed that the physiochemical properties of pulp were greatly affected with addition of chemical preservatives.The combination of preservatives at concentration 2000 ppm is very effective in limiting the microbial growth and the overall preservation of the fruits. It was concluded that with respect to preservation of Avocado slices in *Aloe vera*, the flavor of the fruit was altered but the overall preservation was successful and the combinatorial use of these chemical preservatives in Avocado slices significantly supported in retaining quality of the same for 40 days storage.

Keywords
Aloe vera, Food Preservation, *Persea americana,* Potassium Metabisulphite, Sodium Benzoate, Shelf Life.

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1. INTRODUCTION

Avocado or *Persea americana* is a sub-tropical fruit originating in Mexico. It is a berry which grows on trees and is a popular seasonal fruit which is available for a short span of time. It takes around nine months time to grow and mature as a fruit. It is a good source of potassium and healthy fats and is often referred to as a super fruit.[1] Avocado has a high content of lutein which protects the skin from aging and also keeps the eyes healthy. It is a rich source of vitamins,

minerals, antioxidants, good fats and phytosterol. Research has been going on to study the effect of avocado on diabetes and hypertension and the results so far have been promising. Another research study shows that consumption of avocado leads to improved diet quality and nutrient intake, including a lowered risk of metabolic syndromes.[2]Avocado is high in healthy fats particularly oleic acid which is believed to be associated with reduced inflammation and also have beneficial effects on cancer linked genes.[3] Avocado has the nutritional properties that aid in lowering cholesterol and blood glycemic index/ tri-glyceride levels and increase HDL cholesterol which is the "good cholesterol".[4][5].Vitamins present in Avocado like A, D, E and K are fat soluble along with carotenoids which are anti-oxidants have proved to be help absorb nutrition better in the body. Another study involving addition of Avocado oil to food substances shows to increase antioxidant absorption.[6] Vitamins present in Avocado like A, D, E and K are fat soluble along with carotenoids which are antioxidants have proved to be help absorb nutrition better in the body. Another study involving addition of Avocado oil to food substances shows to increase anti-oxidant absorption.[6] Avocado along with increasing absorption of anti-oxidants is also high in anti-oxidants itself including carotenoids, lutein and zeaxanthin which have proved to be incredibly important for eye health.[7] Keeping the shelf life of these fruits in mind it is essential to develop a technique of preservation using chemical or natural preservatives. Two such chemical preservatives namely sodium benzoate and potassium metabisulphite have been used in different combinations in this experiment which have shown great potential in combating microbial contamination. The mechanism of preservation with Sodium Benzoate starts with the absorption of benzoic acid into the cell. If the intracellular pH falls to 5 or lower, the anaerobic fermentation of glucose through phosphofructokinas e decreases sharply which inhibits the growth and survival of microorganisms that cause food spoilage. The human body rapidly clears sodium benzoate by combining it with glycine to form hippuric acid which is then excreted. The metabolic pathway for this begins with the conversion of benzoate by butyrate-CoA ligase into an intermediate product, benzoyl-CoA, which is then metabolized by glycine N-acyltransferase into hippuric acid.[8] The mechanism of preservation with Potassium Metabisulphate begins when the Bisulfite participates in three important types of reactions with biomolecules, sulfonation (sulfitolysis), autooxidation with generation of free radicals, and addition to cytosine. Products of sulfonation reactions have been shown to be long-lived in vivo and may be highly reactive. Products of auto oxidation are responsible for the initiation of lipid peroxidation, which, could damage plasma membranes and hence controlling the growth of organisms.

The natural preservative used in the study is *Aloe vera. Aloe vera*, from rigvedic times has been known for its medicinal properties majorly used for wound healing, treating burns, minimizing frost bite damage, protection against skin damage from x-rays, lung cancer, intestinal problems, increasing high density lipoprotein (HDL), reducing low density lipoprotein (LDL), reducing blood sugar in diabetics, fighting acquired

immune deficiency syndrome (AIDS), allergies and improving immune system. *Aloe vera* gel has revealed the presence of more than 200 bioactive chemicals.[9] Based on the above mentioned studies and keeping the medicinal properties, nutritional value, application and delicacy of this fruits in mind, an experiment was designed using a combination of two chemical preservatives and a natural preservative in order to extend the shelf life of these two fruits with the aim of retaining its nutritional value and quality at its best so that it can be consumed and utilized outside its season of availability.

2. MATERIALS AND METHODS

2.1. Collection of Fruit sample:

Fresh and Mature Avocados were sampled under standard conditions from the local market. The fruits were even in color, size, appearance and weight. Distilled water was used to remove any unwanted dirt from the exocarp of the fruits. Potassium metabisulphite (K2S2O5) and sodium benzoate (NaC6H5CO2) were purchased from a local store as they are easily available food preservatives. *Aloe vera* was obtained from the premises of the college garden. It was thoroughly washed with distilled water to remove dirt and the gel was obtained from the leaves and preserved in the fridge for further use.

2.2. Slicing of Avocado

Before Slicing, the fruits were washed with distilled water and air dried. By using a sterile stainless-steel knife, the endocarp of the fruit was removed (i.e; the seeds), leaving behind Exocarp and Mesocarp of the fruit. Further the Exocarp was carefully removed and the mesocarp was sliced.

2.3. Processing / Pasteurization of the Samples

The Sliced Avocados were further subjected to pasteurization by placing the beakers containing the samples in a water bath at 82°C for 30 minutes.[11]

2.4. Treatment of the samples with preservatives

75ml of *Aloe vera* gel was obtained and Avocado slices were suspended completely in it for long term preservation. Further, a preservative solution of 75ml for each concentration of the preservative was made of double distilled water and the combination of preservatives i.e; Sodium benzoate and Potassium metabisulphite as given below:

Table 1: Different concentrations of preservatives/ Treatment combinations (mg/ml)

Treatment	Sodium Benzoate (mg/ml)	Potassium metabisulphite (mg/ml)
Control(J1)	-	-
J2	1 mg/ml	1 mg/ml
J3	1.5mg/ml	1.5mg/ml
J4	2mg/ml	2mg/ml
J5	Preservation in *Aloe vera*	

The sliced samples after treatment with preservatives were then transferred into 15 different sterilized Phyta jars containing 75 ml of preservative solution each and 5 jars with *Aloe vera* . These jars were labeled as 1mg/ml, 1.5 mg/ml, 2 mg/ml and Aloe vera respectively. Also, 5 jars with 75 ml of distilled water containing sliced sample was kept and considered as control and was labeled as Control respectively. [10]

3. ANALYSIS OF PHYSICAL APPEARENCE

The samples were accessed on the basis of change in physical aspects like colour/appearance, texture, taste / flavour, aroma/smell.

4. MICRO-BIOLOGICAL STABILITY OF THE SAMPLES

The samples were cultured into agar mediums in order to examine the bacterial and fungal counts. 100 µl of stored pulp and 100 µl of homogenized Avocado slices was pipetted out and spread on to the petri plates using a spreader under aseptic conditions. Petri plates were incubated further at 37°C for 24 hours. In order to determine the bacterial count of chemically preserved Custard Apple pulp, Muller Hinton agar was selected as nutrient source and for fungal count Potato Dextrose Agar was selected. The results were calculated and recorded.[12]

5. PHYSICOCHEMICAL ANALYSIS OF SAMPLES

Various parameters were accessed to check the quality and degree of preservation of the fruit samples. These parameters included measuring the pH using a pH meter, determining the acidity of the samples by titrating the samples against 0.1N NaOH base, determining the reducing sugar content by carrying out Benedict's test and Protein estimation done by Lowery's method. These parameters were carried out following the method of AOAC.

6. RESULTS AND DISCUSSIONS

The aim of this study was to determine the combinatorial effect of the two preservatives used namely sodium benzoate and potassium metabisulphite in Custard Apple and sodium benzoate and potassium metabisulphite along with *Aloe vera* in Avocado as it maintains the microbiological and physiochemical properties of the samples incubated at room temperature for 40 days. The different combinations of food preservative used are as mentioned in table 1 and table 2 respectively. The research concluded that the physiochemical properties and Microbiological control were greatly influenced by the chemical preservation. The observations and results are as follows:

6.1. Quantitative Estimation of Reducing Sugar By Benedict's Test

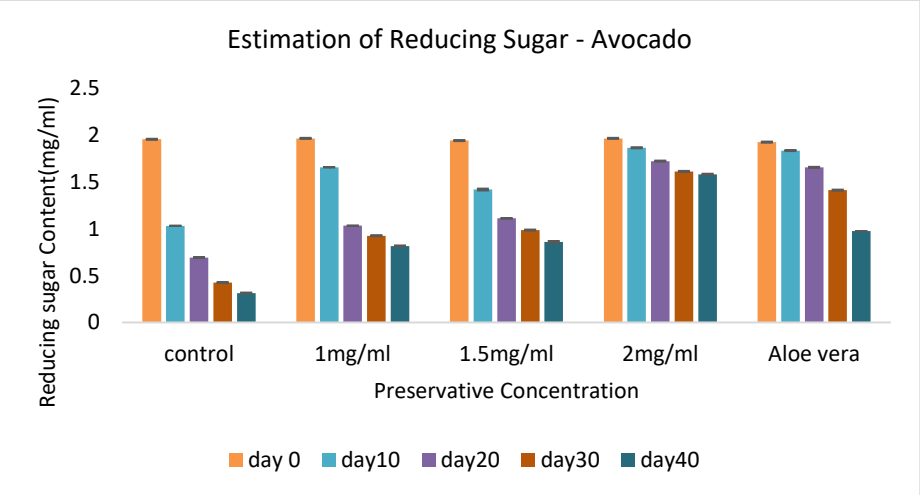

Figure 1: Showing the level of preservation of Reducing Sugars over a period of 40 days

The graph above records the statistics of the reducing sugar content estimated via benedict's test from day 0 to day 40 of preservation. The graph indicates that on the first day treatment, the preservatives exhibited no effect and the content of reducing sugars was the same in all the 5 jars (approximately 1.96mg/ml).However as time progressed the reducing sugar content decreased in tubes 1mg/ml and 1.5 mg/ml with Control showing the least amount of retention (0.31mg/ml). Jar with preservative concentration of 2 mg/ml, however, showed the best results by retaining the reducing sugar content best and closest to that as on day 0 (1.58 mg/ml) followed by the sample preserved in *Aloe vera* (0.97mg/ml).

6.2. Quantitative estimation of Proteins by Lowry's method

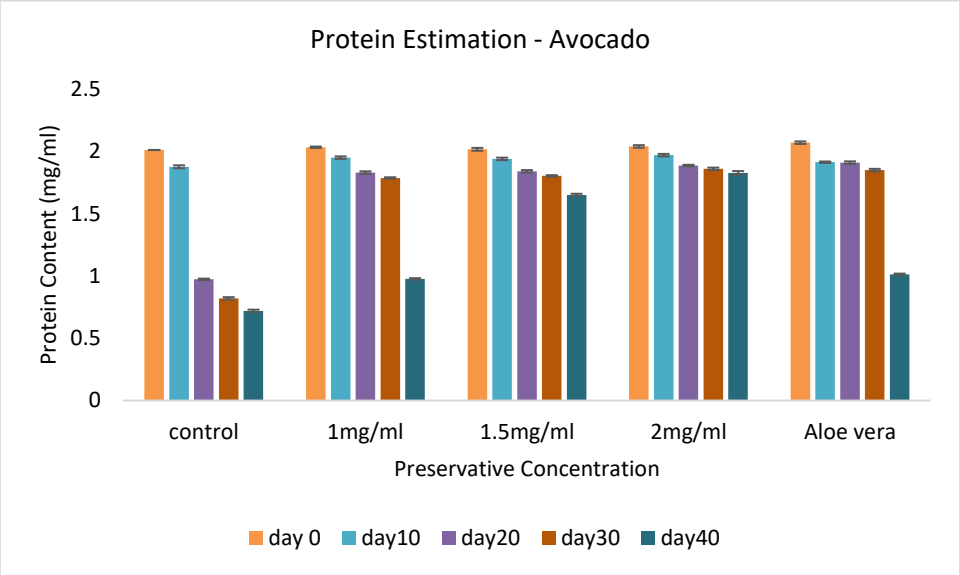

Figure 2: Showing the level of preservation of proteins over a period of 40 days

The graph above records the statistics of the protein content estimated via Lowery's test from day 0 to day 40 of preservation. From the graph it is understood that on the first day treatment, the preservatives exhibited no effect and the content of proteins was the same in all the 5 tubes (approximately 2.0 mg/ml). However as time progressed the protein content decreased drastically in Control (down to 0.72mg/ml) followed by jars preserved with 1mg/ml and 1.5 mg/ml. 2 mg/ml, however, showed the best results by retaining the protein content best and closest to that as on day 0 (1.82 mg/ml). Other than 2 mg/ml, jar with samples preserved in *Aloe vera* showed good results with consideration to preservation (1.01 mg/ml).

6.3. pH retention in Slices of Avocado and Preservative solution.

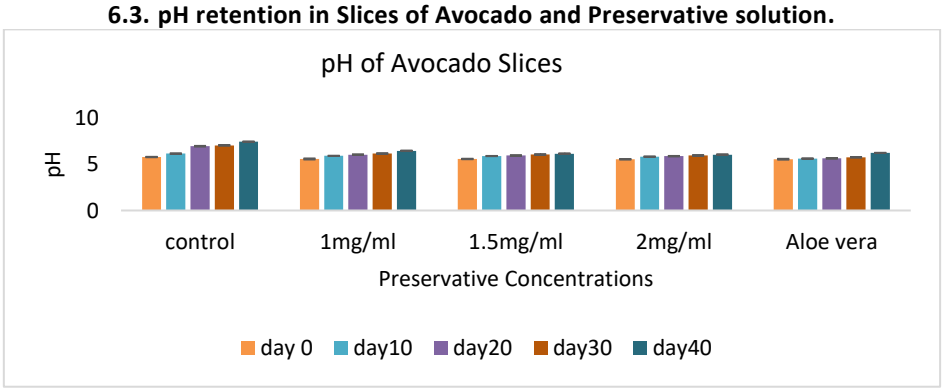

Figure 3: Showing the pH retention of Avocado slices over a period of 40 days

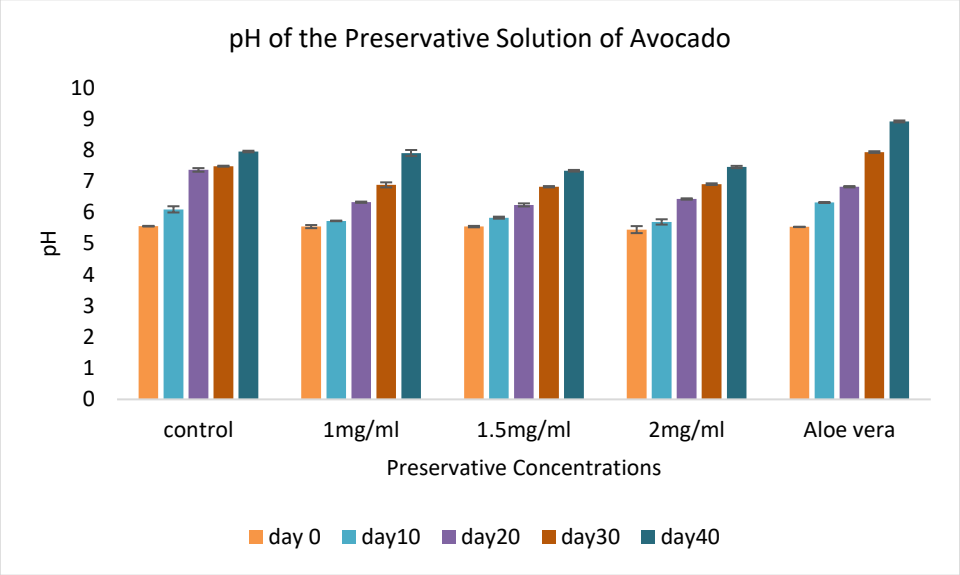

Figure 4: Showing the pH retention of the preservative solution over a period of 40 days

The graphs record the statistics of the pH changes in the preservant solution and the preserved slices from day 0 to day 40 of preservation. It was detected using a pH meter. The pH of the slices and the preservant solution were detected to be different. In case of slices, from the graph it is observed that on the first day treatment, the preservatives exhibited no effect on the pH and was the same in all the 5 jars. However, as time progressed the pH of slices in Control increased becoming alkaline in nature, followed by 1mg/ml and 1.5 mg/ml. 2 mg/ml, however, showed the best results by retaining the pH best and closest to that as on day 0 with slight variations. After 2 mg/ml, jar of samples preserved with *Aloe vera* retained the pH closest to the fresh sample. It was also observed that the pH of the solution increased to a large extend making the solutions highly alkaline yet retaining the properties of the preserved slices. The pH of *Aloe vera* increased with the passage of time but retained the quality of the slices.

6.4. Change in Acidity

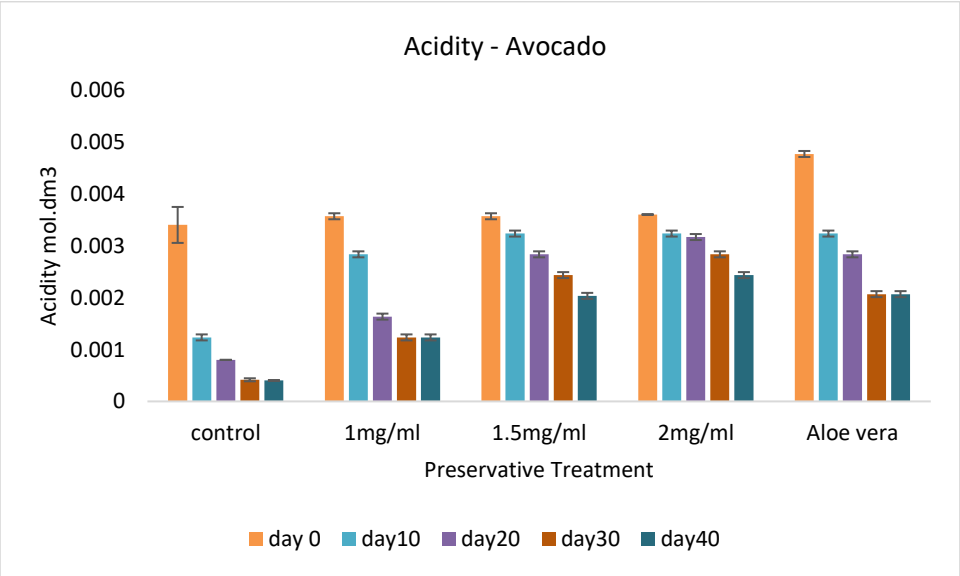

Figure 5: Showing the Acidity Regulation over a period of 40 days

The graph records the statistics for the acidity of the Avocado slices estimated by titrating the sample against 0.1N NaOH from day 0 to day 40 of preservation. The graph indicates that on the first day treatment, the preservatives exhibited no effect and the acidity was approximately 0.0036 mol.dm^3 in all the 5 jars. Jar Aloe vera, however showed a slightly higher acidity reading of 0.0048 which is probably due to treatment with *Aloe vera*. As time progressed, the acidity significantly decreased in jars 1mg/ml and 1.5 mg/ml with Control showing the highest amount of degradation(0.00004mol.dm^3) and least amount of acidity. 2 mg/ml, however, showed the best results by retaining the acidity best and closest to that as on day 0(0.0024mol.dm^3). The jar which preserved the sample in *Aloe vera* showed the same results as jar 1.5 mg/ml.

6.5. Microbial Count

6.5.1Total Bacterial Count (TBC) in *Persia americana*

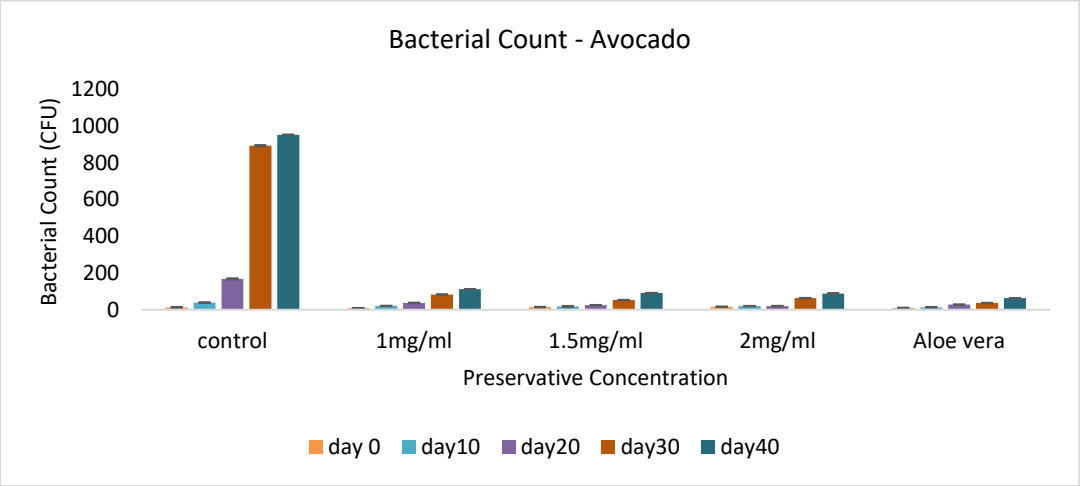

Figure 6: Showing the Bacterial count over a period of 40 days

The graph records the statistics of the Bacterial Count from day 0 to day 40 of preservation. From the graph it is understood that on the first day treatment, the preservatives exhibited no effect on the bacterial count and was the same in all the 5 Jars (upto 12.6 x 10^3 cfu). However as time progressed the bacterial count of control increased drastically (up to 951 x 10^3 cfu), followed by tube 1mg/ml and 1.5 mg/ml. 2 mg/ml along with Aloe vera, however, showed the best results by retaining the bacterial count best and closest to that as on day 0 with slight variations (63.3 x 10^3 cfu). This could be due to the anti-bacterial properties of *Aloe vera*.

6.5.2. Total Fungal Count (TFC) in *Persia americana*

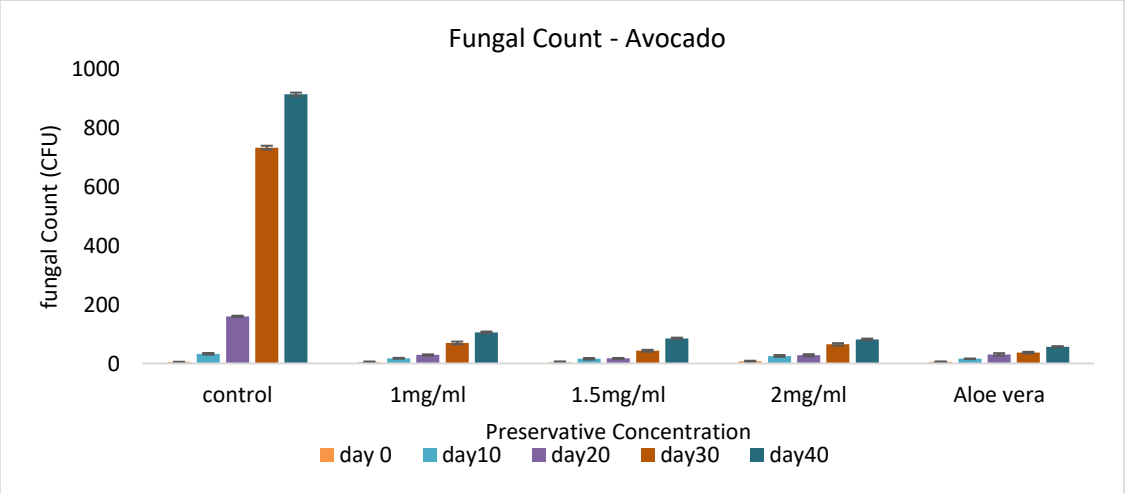

Figure 7: Showing the Fungal count over a period of 40 days

The graph records the statistics of the fungal Count from day 0 to day 40 of preservation. From the graph it is understood that on the first day treatment, the preservatives exhibited no effect on the fungal count and was the same in all the 5 Jars (upto 7.3 x 10^3 cfu). However as time progressed the fungal count of tube Control increased drastically (915 x 10^3 cfu), followed by Jar 1mg/ml and 1.5 mg/ml. 2 mg/ml and Aloe vera, however, showed the best results by retaining the fungal count best and closest to that as on day 0 with slight variations (upto 57 x 10^3 cfu). This is probably due to the anti-fungal properties of *Aloe vera*.

7. CONCLUSION

The proposed study verified the combinatorial of impact of Sodium Benzoate and Potassium Metabisulphite on the physicochemical properties and microbial growth of *Persea americana* (Avocado) along with the effect of preserving in *Aloe vera* of the Avocado slices stored at room temperature for 40 days. From this study it is concluded that both the preservatives (Sodium Benzoate and Potassium Metabisulphite) used in combination of different concentrations of 1000ppm, 1500ppm and 2000ppm each were effective in restricting the microbial growth. It was successful in keeping the 2 samples' characteristics substantially retained hence maintaining its quality attributes. The colour /appearance, texture, taste/flavour, aroma /smell was very well retained in all the concentrations and in *Aloe vera*, whereas in the samples considered as control with no treatment with any preservative was highly deteriorated after 10 days of preservation. Among the proposed concentrations, 2000ppm was the most effective in retaining the properties of the samples as compared to the other concentrations and therefore is recommended to combat spoilage in these particular samples and maintaining its attributes and physicochemical properties. Also, preservation of Avocado slices in *Aloe vera* comes second in preservation after preservative concentration of 2000ppm. However, *Aloe vera* tends to alter the flavor of the sample to leave a slight bitter aftertaste. Even then, from a survey conducted with 100 participants who were asked if they preferred natural preservative of *Aloe vera* over chemical preservative even though it alters the flavor and leaves a bitter aftertaste, it was concluded that 52% of the participants prefer the natural preservative over the chemical one.

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