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A Study to Combat Microbial Food Spoilage Activities in Custard Apple (*Annona Reticulata)* Using A Combination Of Chemical Preservatives

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ABSTRACT

Annona reticulata is a seasonal fruit available for a short period of time with a shelf life of three to six days. Its shelf life can be increased upon refrigeration up to few more days. However, an effective method of preserving any food item is by the use of preservatives. This method ensures retaining the quality, chemical composition, texture and colour of the fruits. In this research work, Custard Apple pulp were stored at room temperature to study the combinatorial effect of the two chemical preservatives (Sodium Benzoate and Potassium Metabisulphite). The chemical preservatives were used in a fixed ratio with increasing degree of concentration to check which is the most effective combination in preserving the fruits. The physiochemical properties and Microbial growth 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 the combinatorial use of these chemical preservatives in Custard Apple pulp significantly supported in retaining quality of the same for 40 days storage.

***Keywords*:** *Annona reticulata, Food Preservation, Potassium Metabisulphite, Sodium Benzoate, Shelf Life.*

1. INTRODUCTION

Custard apple or Annona reticulata [1], a tropical fruit, is a small shrub of family Annonaceae of genus Annona. It is a tropical fruit and its place of origin is still being debated. The fruit is soft and chewy with a hard exocarp. The mesocarp is white in color and has a creamy texture. It can be consumed just by itself or in processed forms of shakes, smoothies, deserts and ice cream. The fruit is an excellent alternative to dairy products, thus making it a very efficient replacement of dairy products to people who are allergic to it. This seasonal fruit and its extracts/active constituents have proved in several studies and reports to have anti-oxidative, anti-inflammatory and analgesic properties. The fruit consists of high levels of vitamin A making it an ideal choice for maintaining healthy skin, hair and better eyesight. It is also used as a moisturative and anti-aging agent. The pulp can be used as a balm to treat boils and ulcers. Custard apple has been used quiet efficiently in Ayurveda and has multiple medicinal properties. For instance, its anti-hyperglycaemic effect can prevent diabetes to get worse. [3] Custard Apple have tannins and flavonoids which are known phytochemicals. These healthy compounds are linked to reduce lipid levels and blood pressure. In research studies flavonoids have shown strong tendencies of lowering heart diseases. [4] In a study conducted where the compounds of the fruit were isolated (about 14 compounds), 16,17-dihydroxy-entkauran-19-oic acid showed significant activity against HIV replication in H9 lymphocyte cells with an EC50 value of 0.8 µg/mL [7]. As known the shelf life of custard apple is very short, a technique of preservation of this fruit was designed using a combination of artificial preservatives namely sodium benzoate and potassium metabisulphite have been used in different combinations 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 phosphofructokinase 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.[5] The mechanism of preservation with Potassium

Metabisulphite 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. 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 Custard Apples 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 ($K_2S_2O_5$) and sodium benzoate ($NaC_6H_5CO_2$) were purchased from a local store as they are easily available food preservatives.

2.2. Pulping of Custard Apple

Before extraction of the pulp, 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 of both the sections and the Custard Apples were pulped using a hand blender (Phillips model HR1602/00). [6]

2.2. Processing / Pasteurization of the Samples

The pulped Custard Apples were further subjected to pasteurization by placing the beakers containing the samples in a water bath at 82℃ for 30 minutes.[6]

2.4. Treatment of the samples (pasteurized pulp of Custard Apple) with preservatives

As the temperature of pulp decreased to room temperature the preservatives i.e; Sodium benzoate and Potassium metabisulphite at different concentrations were added collectively under aseptic conditions. The pulp samples after treatment with preservatives were then transferred into 20 different sterilized test tubes containing 20 ml each. These test tubes were labelled as T2, T3 and T4 respectively. Also, 5 tubes with 20 ml of non-preservative treated pulp was kept and considered as control and was labelled as T1 respectively.[6]

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.RESULT AND DISCUSSION:

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 as it maintains the microbiological and physiochemical properties of the samples incubated at room temperature for 40 days. The research concluded that the physiochemical properties and Microbiological control were greatly influenced by the chemical preservation. The different combinations of food preservative are used in increasing concentrations [6] and are as mentioned in table 1 as follows:

Treatment	*Sodium Benzoate (mg/ml)*	*Potassium metabisulphite(mg/ml)*
T1	-	-
T2	1	1
T3	1.5	1.5
T4	2	2

Table 1: Combination of chemicals used

6.1. Retention of Reducing sugars Content

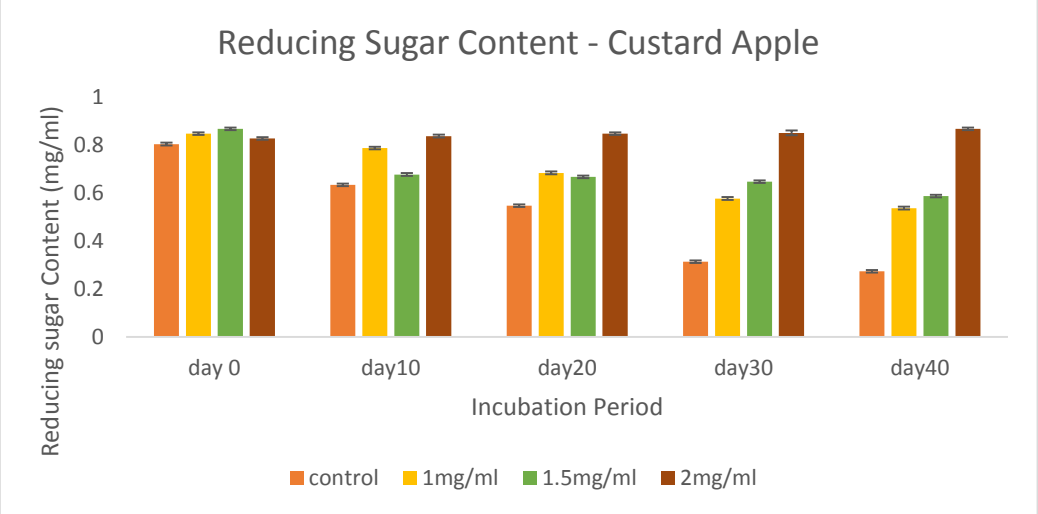

Figure 1: Showing the level of preservation of Reducing Sugars over a period of 40 days

Figure 1 records the statistics of the reducing sugar content estimated via benedict's test from day 0 to day 40 of preservation. From the table and graph it is understood that on the first day treatment, the preservatives exhibited no effect and the content of reducing sugars was the same in all the 4 tubes. However, as time progressed the reducing sugar content decreased in tubes T1, T2 and T3 with T1 showing the least amount of retention followed by T2 and T3. T4, however, showed the best results by retaining the reducing sugar content best and closest to that as on day 0.

6.2. Retention of Proteins Content

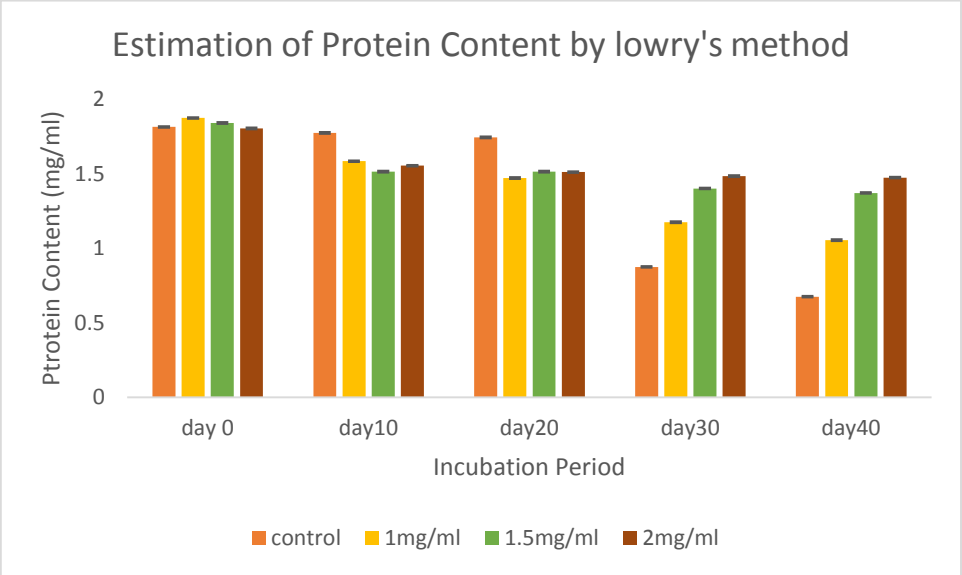

Figure 2: Showing the level of preservation of proteins over a period of 40 days

Figure 2 records the statistics of the protein content estimated via Lowery's test from day 0 to day 40 of preservation. From the table and 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 4 tubes. However, as time progressed the protein content decreased drastically in tube T1, followed by tube T2 and T3. T4, however, showed the best results by retaining the protein content best and closest to that as on day 0 with slight variation.

6.3. pH Retention

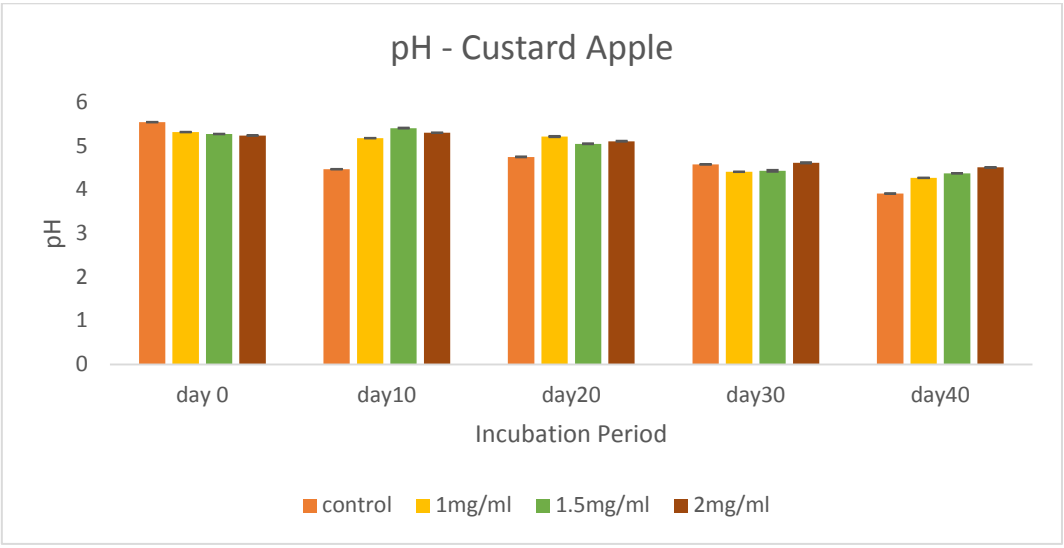

Figure 3: Showing the pH retention of Custard Apple pulp over a period of 40 days

Figure 3 records the statistics of the pH changes from day 0 to day 40 of preservation. It was detected using a pH meter. From the table and graph it is understood that on the first day treatment, the preservatives exhibited no effect on the pH and was the same in all the 4 tubes. However, as time progressed the pH of tube T1 decreased drastically, followed by tube T2 and T3. T4, however, showed the best results by retaining the pH best and closest to that as on day 0 with slight variations.

6.5. Acidity

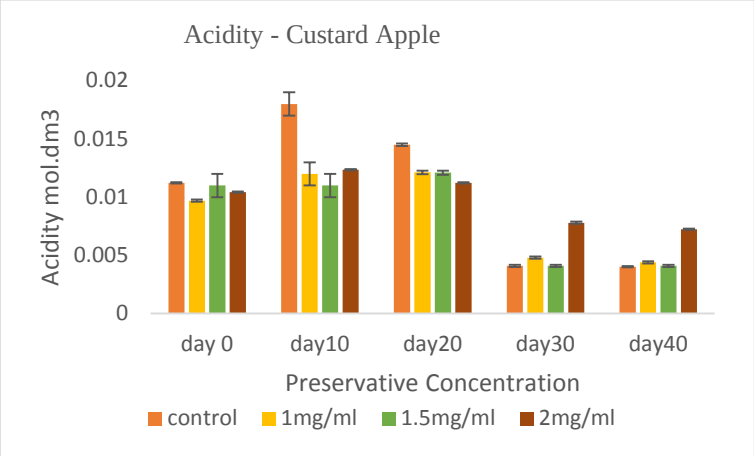

Figure 4: Showing the Acidity Regulation over a period of 40 days

Figure 4 records the statistics for the acidity of the pulp samples estimated by titrating the sample against 0.1N NaOH from day 0 to day 40 of preservation. The table and graph indicate that on the first day treatment, the preservatives exhibited no effect and the acidity was approximately 0.1 mol.dm^3 in all the 4 tubes. However, as time progressed, the acidity significantly decreased in tubes T1, T2 and T3 with T1 showing the highest amount of degradation followed by T2 and T3. T4, however, showed the best results by retaining the acidity best and closest to that as on day 0.

6.6. Microbial Count

6.6.1. Total Bacterial Count (TBC)

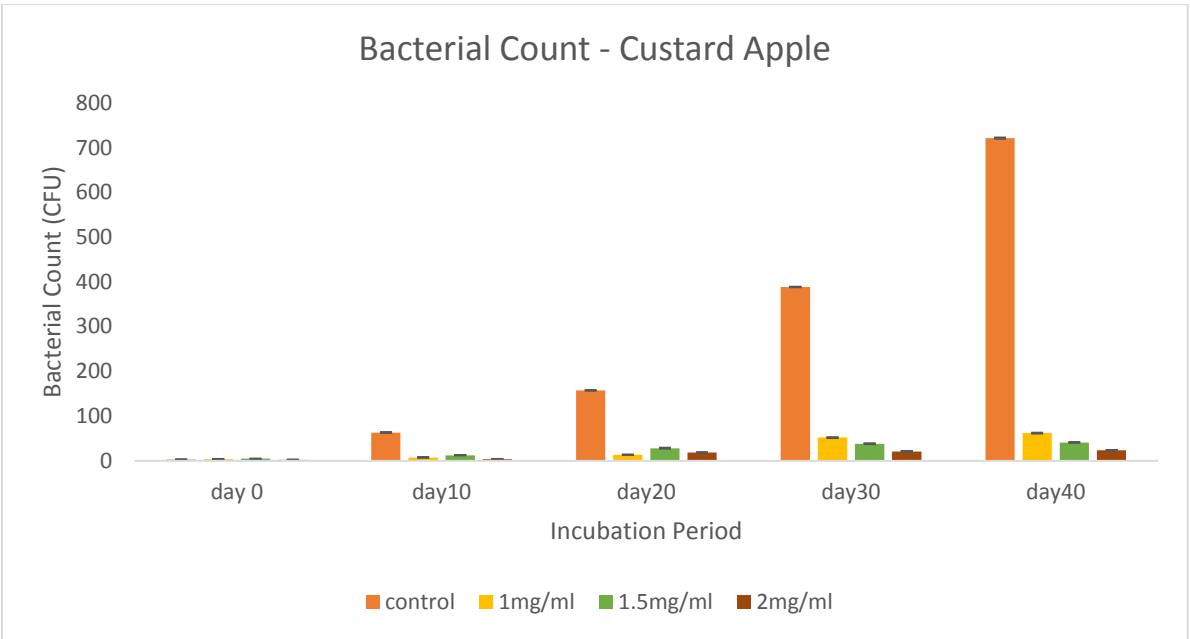

Figure 5: Showing the Bacterial count over a period of 40 days

Figure 5 records the statistics of the Bacterial Count from day 0 to day 40 of preservation. From the table and 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 4 tubes. However, as time progressed the bacterial count of tube T1 increased drastically, followed by tube T2 and T3. T4, however, showed the best results by retaining the bacterial count best and closest to that as on day 0 with slight variations.

6.6.2. Total Fungal Count (TFC)

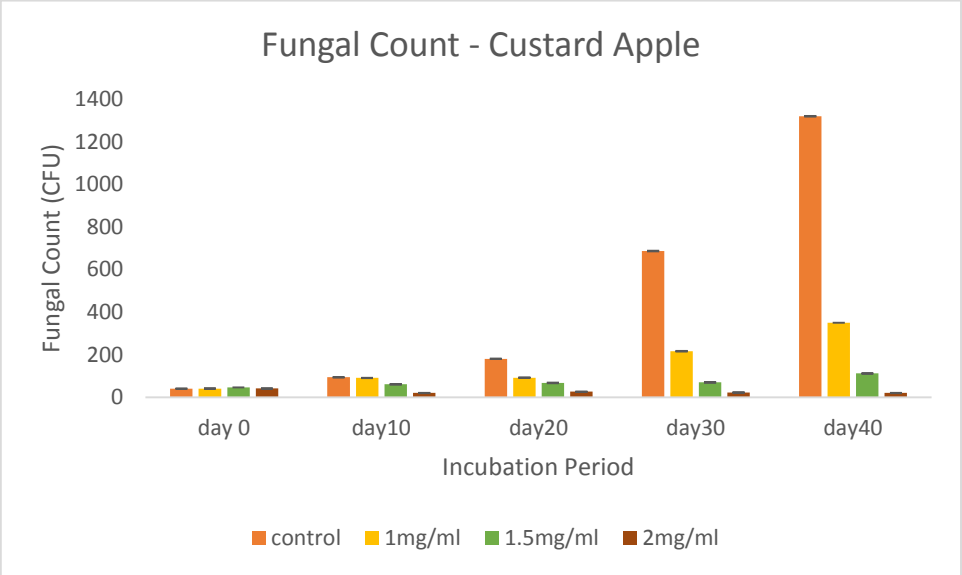

Figure 6: Showing the Fungal count over a period of 40 days

Figure 6 records the statistics of the fungal Count from day 0 to day 40 of preservation. From the table and 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 4 tubes. However, as time progressed the fungal count of tube T1 increased drastically, followed by tube T2 and T3. T4, however, showed the best results by retaining the fungal count best and closest to that as on day 0 with slight variations.

7.CONCLUSION

The proposed study verified the combinatorial of impact of Sodium Benzoate and Potassium Metabisulphite on the physicochemical properties and microbial growth of *Annona reticulata* (Custard Apple) 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 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**,** 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.

REFERENCES

1. Bakane P.H., et al., (2016) Study on Ripening of Custard Apple Fruit (Annona squamosa L.). International Journal of Agriculture Sciences, ISSN: 0975-3710 & EISSN: 0975-9107, Volume 8, Issue 42, pp.-1844-1846.

2. Jianwei Chen, Xiang Li Beneficial Aspects of Custard Apple (Annona squamosa L.) Seeds in Health and Disease Prevention, 2011,Pages 439-445.

3. Rahman, SK. Mizanur. (2011). Antihyperglycemic Studies with Methanol Extract of Annona reticulata L. (Annonaceae) and Carissa carandas L. (Apocynaceae) Leaves in Swiss Albino Mice.

4. Boyer J, Liu RH. Apple phytochemicals and their health benefits. *Nutr J.* 2004; 3:5. Published 2004 May 12. doi:10.1186/1475-2891-3-5.

5. H A Krebs, D Wiggins, M Stubbs, A Sols, and F Bedoya, Studies on the mechanism of the antifungal action of benzoate, *Biochem J*. 1983 Sep 15; 214(3): 657–663.
6. Razzaq A, Imtiaz A, Junaid MA, et al. Strategies to Combat Microbial Food Spoilage Activities in Peach Pulp. Electronic J Biol, 13:4.
7. MR Suchitra1 and S Parthasarathy, Sitaphal: Reemergence, Research Journal of Pharmaceutical, Biological and Chemical Sciences, ISSN: 0975-8585, May – June 2015, Page No. 1560.
8. Hussain A, Azhar H, Bilal A, et al. (2014). Analysis of physicochemical stability of apricot pulp stored with chemical preservatives. *Int J Novel Res Life Sci.* 1: 17-23.
9. Akhtar, Saeed & Riaz, Muhammad & Ahmed, Anwaar. (2010). Physico-chemical, microbiological and sensory stability of chemically preserved mango pulp. Pakistan Journal of Botany. 42. 853-862.

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