A STUDY ON SHELF LIFE OF FOOD THROUGH SMART PACKAGING USING NANOTECHNOLOGY

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Introduction

Nanoparticles (NPs), a broad category of materials, are granules with at least 1 to 100 nanometers (nm). Much curiosity is generated by the distinctiveness of nanoparticles in terms of their chemical and physical properties. The properties of nanoparticles with increased damping, mechanical stability, superior thermal conductivity, and stronger strength are distinctive features. Due to its use in optical electronics, sensing technology, food and food packaging, metal-oxide nanoparticles with excess surface area have garnered a lot of scientific interest. Nanomaterials' exceptional chemical stability, magnetic properties, and biocompatibility have drawn the attention of numerous researchers. Antibacterial, antifungal, antioxidant, and anticancer properties of nanomaterials.

Processing Method of Nanoparticles: There are two methods for synthesising nanomaterials. First, there is the "bottom up" approach, in which smaller structures are created and then put together. Morphology, crystallinity, particle size, and chemical composition are the primary control factors. Examples include colloidal fusion, self-emission, laser ablation, and chemical synthesis. and second, a "top-down" approach in which attributes of huge things are changed for smaller ones. Examples include the deposition and development of films, nanoprinting/lithography, the etching method, mechanical polishing, etc

Apple and Nanoparticles: The apple (*Malus demestica cv. Borkh*) is famous as the "King of Leafy Fruits" because of beautiful shape, attractive color and very delicious taste. The apple, the world's first edible fruit, belongs to the Rosaceae family and the Pomoidae subfamily. Apples are usually harvested in a short period of time span, so fruit storage is important to facilitate marketing and deliver quality products to fresh and processed stores. Due to the lack of year-round processing and land transportation, most of the apples go to waste. There is a lot of fruit in the market during harvest time. Today, farmers are not getting a fair price, so maintaining the durability of apple is necessary for the benefit of farmers and consumers. Apples and apple juice which are less processed have uncovered skin and cell walls and liquid materials which are prone to contamination by airborne & environmental microbes. Fresh fruit and vegetable juices spoil very quickly, resulting in a deterioration of organoleptic and physico-chemical parameters, due to which the consumer refuses the product. Fresh juices pressed from fruits and vegetables are easily damaged and no longer shelf life. An increase in pH value of juice is optimal for the growth of thermophilic bacteria. The juice degrades and spoils as a result of an accumulation of metabolic by products brought on by an increase in the bacteria

loads. The shelf life of the product is shortened by the destruction of numerous crucial nutritional components, including as antioxidants, vitamins A, C, and E, and phytonutrients, during storage under the effect of light and various temperatures. Customers reject the goods because of sensory factors including colour, texture, or appearance that lead the product to spoil. The microbial and non-microbial contamination that happens during processing is not sufficiently accounted for, despite the fact that washing can reduce microbial load to some amount. Coating with a preservative is one method for extending the shelf life of apples and apple juice.

Guava and Nanoparticles: Guava is promoted as a fruit that is "health helpful" and has a lot of vitamin A and C. Guava is a potent fighter against free radicals and oxidation, which are responsible for many degenerative disorders due to the high concentration of vitamin C (ascorbic acid) in it. The major causes of the greatest quantity of guava being wasted in the fruit processing business are high domestic production, high perishable parameters, and a lack of proper technology. Guava and guava juice's colour, aroma, texture, and flavour were all enhanced by the use of food additives and preservatives for the advantage of the food business. Vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols, and triterpenoid acids make up the majority of guava's nutritional components. Numerous guava health advantages have been linked to these and other substances. Guava (*Psidium guajava L.*) is a famous seasonal fruit with climatic characteristics. It has a relatively short lifespan (3–4 days) at tropical room temperature (28 ± 2˚C) due to physiological characteristics, disturbances, postharvest infections and aging. Many factors can affect the stability of guava, such as temperature and relative humidity of storage, packaging material used for guava and type of coating applied. Guava cultivator store guava in traditional packaging like paper/plastic materials. Although these packaging materials have various advantages, they can cause serious ecological issues because they are non-recyclable and non-edible resources. Physiologically, guava is a climate fruit with high respiration and transpiration, similar to various commodities such as bananas and mushrooms. This requires the development of new techniques to extend shelf life, provide better storage conditions, and improve imaging properties

The present investigation "**A Study on Shelf Life of Food through Smart Packaging using Nanotechnology**" was carried out in BBAU Lucknow, to investigate the shelf life of apple, guava, apple juice and guava juice by using Nanoparticles (ZnO, CuO, MgO) synthesised Via

sol-gel method by using different parameter (Proximate analysis, Physiochemical, Microbiological analysis, Morphology and overall acceptability. The fruits were collected from the local market of Lucknow. The experiment was conducted for 3.5 years i.e. on 2018 & 2022 with the main objectives to extend the storage life of all treatments at room temperature.

The research was conducted by using following objectives:

1. **To study about nanopackaging material used in food packaging**
2. **To Study about Synthesis of Nanoparticles via sol-gel method and its characterization by using various technique**
3. **To prepare edible coating by using edible wax and nanoparticles and application of nanoparticles on sample**
4. **To study the shelf life and Microbial activity of sample during storage period**
5. **To study the sensory and phytochemical analysis of sample during storage period.**
6. **To introduce the different types of smart packaging used in nanotechnology.**

Hypothesis:

i. $H_0$1: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on TSS value of all treatment.

ii. $H_0$2: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on pH Value of all treatment.

iii. $H_0$3: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Acidity Value of all treatment. iv. H04: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Specific Gravity of all treatment.

iv. $H_0$5: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Reducing sugar of all treatment.

v. $H_0$6: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on water content of fruit sample.

vi. $H_0$7: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Microbial Count of all treatment.

vii. $H_0$8: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on overall acceptability of all treatments

Methodology:

Locale of the Study: - The experiment was carried out in the research laboratory of the Department of Food and Nutrition, Physics, Chemistry, Microbiology and USIC Lab of Babasaheb Bhimrao Ambedkar University Lucknow.

Period of study: - The research was place over a 3 and half year period. The entire research was broken down into phases, and each task was carried out in a scheduled manner in a different phase, one after the other.

a) Preparatory Stage: The study's first stage was devoted to gaining a thorough understanding of the subject matter. In order to construct the experiment protocol, subject matter experts were contacted. b) The Experimental Phase: During this stage, experiments were conducted that involved the production of nanoparticles, their characterization using various techniques, their application to fruits, and the evaluation of their shelf life using various metrics.

Variables: A variable is a property that could alter in a research study and is often one that could influence or reflect a relationship or outcome. In this study, there are two factors:

a) Independent Variables: An independent variable in a research is subject to control or other manipulation by the researcher. In order to establish the relationship between the independent and dependent variables, a researcher will purposefully change one independent variable while watching to see if and how the dependent variable responds. Days of storage time and temperature were independent variables in this study.

b) Dependent Variables: A measurement that represents an outcome in an experiment is referred to as a dependent variable. The researchers have no direct control over this variable. Instead, they seek to learn more about the interactions between different variables by observing how the dependent variable behaves in various circumstances. Dependent Variables in this study were "Nutritional component, Physiochemical parameters, Microbiological parameters, Sensory parameters, phytochemical parameters, Antibacterial activity.

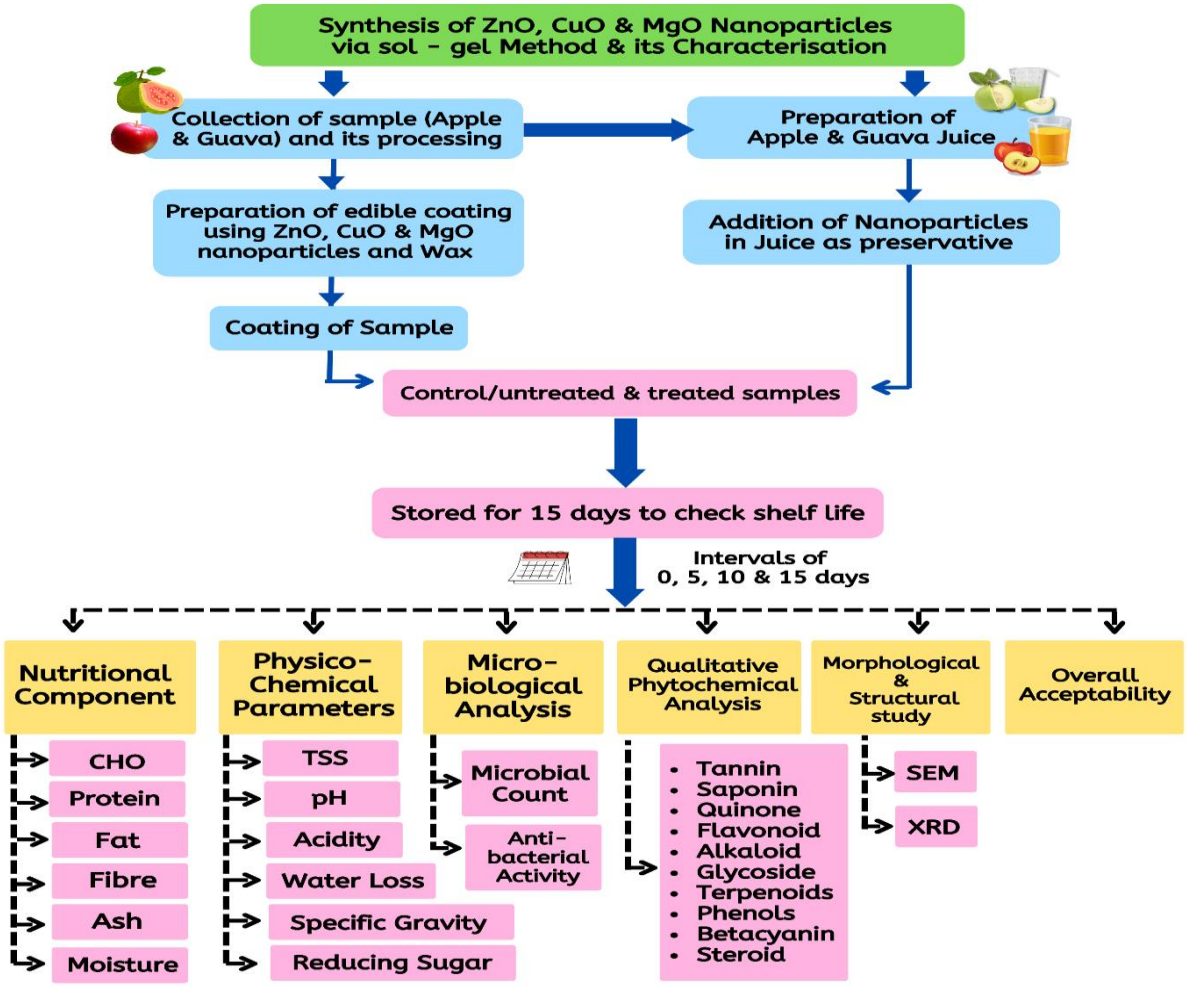

Fig: Systematic diagram of research

Statistical Analysis:

The researcher can make sense of quantitative data with the use of statistical analysis. Quantitative data would be a disorganised mass of numbers without statistics. Researchers can convey, analyse, arrange, assess, and interpret numerical data using statistical methods**.**

At various intervals, one-way ANOVA for the physiochemical test was followed by the application of the t-test to compare the means of the physicochemical parameters. Three replications of each sample- MgO NPs, ZnO NPs, CuO NPs, and untreated/control fruit and juice were used in the experiment, which was carried out using an entirely randomised factorial design. The data was entered onto an excel page with different items/variables listed in columns and subjects listed in rows. Excel 2016 was also used for data analysis. Using mean, standard deviation (SD), and ANNOVA, the data from all treatments were compiled. Using IBM SPSS Statistical Analysis Software, version 24, one-way analysis of variance (ANOVA) was used to evaluate the data. The level of significance was defined as $p \leq 0.05$.

The formulae for mean and standard deviation are given below:

1. **Mean:** The individual observations were tallied up and then divided by the total number of observations to find the mean. The Σ symbol stands for the summation or adding together action.
2. The symbol X stands for the individual observation, n for the total number of observations, and $\bar{x}$ for the mean.

$$\overline{X} = \frac{\Sigma X}{\text{No. of observations (n)}}$$

3. **Standard Deviation:** It is denoted by the Greek letter σ.

$$\sigma = \sqrt{\frac{\Sigma (X - \overline{X})^2}{n}}$$

where, σ = Standard deviation

Σ = summation (x - x) = Square of deviation of each value From the arithmetic mean

f = frequency

n = total number of observation.

4. **Level of significance**: "p" is level of significance

p > 0.05 Not significant

p < 0.10 Marginally significant

p < 0.05 Significant

p < 0.001 Highly significant

There were altogether 16 treatments, which were mention below

Treatments	Sample preparations
G0	**Guava treated with wax**
G1	**Guava treated with ZnO Nanoparticle**
G2	**Guava treated with CuO Nanoparticle**
G3	**Guava treated with MgO Nanoparticle**
G4	**Untreated Guava Juice**
G5	**Guava Juice treated with ZnO Nanoparticle**
G6	**Guava Juice treated with CuO Nanoparticle**
G7	**Guava Juice treated with MgO Nanoparticle**
A0	**Apple treated with wax**
A1	**Apple treated with ZnO Nanoparticle**
A2	**Apple treated with CuO Nanoparticle**
A3	**Apple a treated with MgO Nanoparticle**
A4	**Untreated Apple Juice**
A5	**Apple Juice treated with ZnO Nanoparticle**
A6	**Apple Juice treated with CuO Nanoparticle**
A7	**Apple Juice treated with MgO Nanoparticle**

The results obtained during the investigation have been summarized briefly in the following Headings.

5.1. Synthesis of Nanoparticles (ZnO, CuO and MgO) via sol gel method and its conclusion

5.2. Changes Nutritional composition/ proximate analysis of all treatments during storage period.

5.3. Changes in physiochemical attributes of all treatment during storage period.

5.4. Microbial analysis of all treatments during storage period.

5.5 Qualitative analysis of phytochemical attributes of all treatments during storage period.

5.6 Overall acceptability of all treatments during storage period.

5.7 Changes in structure and morphology of all treatments at initial days and final days.

5.1. Synthesis of Nanoparticles (ZnO, CuO and MgO) via sol gel method and its conclusion

ZnO, CuO and MgO nanoparticles had been effectively synthesized via way of means of easy Sol-Gel approach. The organized nanoparticles have been characterised by the use of XRD, FT-IR, SEM, EDX techniques. The average particle size of CuO, ZnO and MgO is calculated as 61.25nm, 21.82nm and 19.12nm respectively via way of means of the use of Scherer's equation of substance dried at 80°C. The evaluation of FTIR spectrum of CuO indicates peaks at 602.09, 678.39, and 730.19cm^{-1} which consult with the formation of CuO. FTIR spectrum of pure ZnO nanoparticles, the height of peak at 594.56 cm^{-1} changed into the function absorption of Zn-O bond. The peak at 1121.47 cm^{-1} is due to adsorption of CO_2, whereas peaks at 1001.79 cm^{-1} attributes to different Mg-O-Mg vibrations mode of Mg. A SEM image indicates the good agglomeration of CuO nanoparticles. Zinc Nitrate [$Zn(NO_3)_2$] has been used in synthesis process of ZnO with spherical shape via Sol-Gel Method EDX analysis confirm the present of CuO, ZnO and MgO nanoparticles with less impurities and nearly about stoichiometry shelf existence of the product. The approach has an excessive yield and may be used for big scale synthesis of ZnO, CuO and MgO Nanoparticles.

5.2 Changes in Nutritional composition/ proximate analysis of all treatments during storage period.

The critical examination of the data during storage period revealed that the nutritional value was not much affected during the storage. The apple and guava both contain a low amount of protein and fat but good source of carbohydrate. During the storage period the only the parameter which effect mostly was moisture content. The maximum loss in moisture content was recorded in untreated sample i.e., 69.23±0.22(G0), 70±0.44 (G4), 76±1.21 (A0), 79.2±0.29 (A4). The apple and guava both contain a low amount of protein and fat but good source of carbohydrate and fibre. The maximum fibre content found in G1 (5.21g) and the minimum fibre content found in A4 (0.52g).

5.3. Changes in physiochemical attributes of all treatment during storage period.

The total soluble solids increased during storage up to the 15th day and observations for TSS were taken on every 5th day. The mean TSS value of G0, G1, G2, G3 at 0 days were 12°, 12.04°, 12.06°, 12.05° Brix respectively and G4, G5, G6, G7 were 14.1°, 14.1°, 14.2°, 14.09° Brix respectively and the TSS value of A0, A1, A2, A3 were 14˚, 14.03˚, 14.05˚, 14.05˚ Brix and A4, A5, A6, A7 were 17.4°, 17.39°, 17.4°, 17.4° Brix respectively. At 5th day of storage the TSS value in G5 and G7 remained constant but in other treatments increase in TSS content was observed. Maximum TSS in guava fruit was found G0 (12.9°Brix) and minimum in G1 (12.4°Brix), in apple fruit maximum value was found in A0 (15.2°Brix) and minimum in A1 (14.6°Brix) at 15th day of storage period. TSS value in juices of control sample was increased very rapidly and recorded maximum value in G4 (14.9°Brix) and A4 (18.5°Brix) as compared to treated sample which recorded minimum in G5 (14.6°Brix) and A5 (17.9°Brix) at 15th day of storage.

The critical examination of the data during storage period revealed that the rate of decrease in pH of sample G1, G2, G3 started from 4.1 during the initial day to 3.8, 3.7, 3.6 respectively on the 10th day of storage and this value decreased to 3.5, 3.3, 3.4 respectively till the last day i.e. 15th day in treated sample. The rate of decrease of pH in A1, A2, A3 started from 3.5 during the initial day and on 15th day of storage it was observed the value decrease up to 2.9, 3.0, 2.8 respectively. Likewise, in juice sample the pH level of sample at the initial stage was recorded

in G4, G5, G6, G7 is 4.5 and in A4, A5, A6, A7 is 4.2 which decreased up to 3.5 (G4), 4.0(G5), 3.9(G6), 3.8 (G7) and 3.2 (A4), 3.6 (A5), 3.4 (A6), 3.5(A7), after 15 days of storage.

The data indicates that gradual decrease in water content. During the storage period, in guava fruit the maximum water loss was observed in G0 (89%) which was untreated sample and minimum was recorded in G1 (94%) which was treated sample on the 15th day when the investigation was terminated. In pooled data analysis, sample under control or untreated sample exhibited maximum water loss [G0 (89%) and A0 (87%)] on the last day of investigation.

The critical examination of data revealed that during the storage period, the initial ascorbic acid in G0, G1, G2, G3 was 0.421, 0.419, 0.420, 0.421 mg/gm respectively which went on increasing and finally on 15th day of storage when the investigation was terminated at the end of storage maximum ascorbic acid i.e. 0.510 mg/gm was recorded in G0 (untreated sample) closely followed by treated sample. Initially the ascorbic acid content in A0, A1, A2 and A3 were 0.381, 0.380, 0.379, 0.380 mg/gm respectively which increased up to 0.493 mg/gm, 0.432 mg/gm, 0.442 mg/gm and 0.450 mg/gm respectively on 15^{th} days of storage period. In juice sample the maximum ascorbic acid was found in 0.810 mg/ml (A4) and 0.830 in (G4).

During the study, it was observed that up to the fifth day the specific gravity remained almost unchanged in treated sample. However, after 5^{th} day of storage marked variation was observed among different treatments and control. On 10^{th} day there was a sudden fall in specific gravity of fruits in control G0 (0.90) and A0 (0.88) followed by treated sample G1(0.95), G2 (0.94), G2 (0.93), A1 (0.92), A2 (0.91) and A3 (0.90). During the concluding day of the experiment i.e. on the 15th day, minimum specific gravity in fruits sample were (0.86) and (0.85) recorded in control G0 and A0 respectively and in juice sample the minimum specific gravity in fruits sample were (1.079) and (1.035) recorded in control G4 and A4 respectively.

A perusal of data during storage period revealed that there was an increasing trend of reducing sugar up to 10^{th} day of storage and it remained constant in majority of the treatments up to 15^{th} day. The increasing trend was very rapid in untreated sample i.e., G0, G4, A0, A4. The maximum reducing sugar was noticed in untreated guava juice 5.212(G4) and Minimum reducing sugar was noticed in 2.84 (G1) at final days of storage life. Statistical analysis of data revealed that treatments sample treated with ZnO NPs were significantly superior to other treatments and control.

5.4. Microbial analysis of all treatments during storage period.

During the initial days of storage, no microbial was found on the surface of samples but the changes started from 5th days of storage which increased up to 15th days of storage. The maximum microbial count was found in untreated sample i.e., G0 (1.20×10^2), G4 (1.31×10^2), A0 (1.29×10^2), A4 (1.62×10^2). This study shows that the nanoparticles and storage days have a significant ($p\leq0.05$) effect on the growth of micro-organisms. The microbial loads of the in sample treated with ZnO NPs was generally low in comparison to sample treated with CuO and MgO NPs.

Zone of inhibition in tested sample are, *Escherichia coli* and *Staphylococcus aureus*. On the concluded day of study, growth of *staphylococcus Aureus* was minimum in A1(5±0.01) and the growth of *E-Coli* was also minimum in A1 (7±0.04). In all treatments zone of inhibition of *E-Coli* was maximum as compared to *Staphylococcus aureus.* Zone of inhibition of *Staphylococcus aureus* ranges between 5 to 15 mm and Zone of inhibition of *E-Coli ranges* between 7 to 22 mm.

5.5 Qualitative analysis of phytochemical attributes of all treatments during storage period.

Tannins and Terpenoids were present in large amount in Guava fruit and guava juice sample at initial days but as the storage period started increasing, its quantity started decreasing and on the last day it is present in very less amount. In initial days' alkaloids and phenols were present in huge amount in all treatments which was decreases by increasing the storage period and present in very less amount at 15 days. Saponin and Flavonoids were present in huge amount in apple and apple juice treatments in initial days but on final day it presents in little amount. During the it was found that quinone, Betacyanin and steroids were absent all guava sample but Betacyanin was also absent in apple sample. initially quinone was present in apple sample but on last day it was noticed that quinone was absent in apple sample.

5.6 Overall acceptability of all treatments during storage period.

A perusal of data during storage period revealed that the overall acceptability of untreated sample was decreased by increasing the storage period. In comparison to fruit, the

sensory characteristic of juice was not accepted at 15th day of storage. From initial day to 10th day of storage period the sensory acceptability was found to be 'like moderately'.

5.7 Changes in structure and morphology of all treatments at initial days and final days.

The morphology of apple sample at 0 days and 15 days and shows that the apple coated with ZnO NPs +Wax & MgO NPs + wax had a smooth area and the sample treated with CuO NPs had crack in outer layer in sample after 15 days of storage. Figure 4.29 (a) and 4.29 (b) present the guava sample stored at 0 days and 15 days and shows that the guava coated with nanoparticles had a smooth area as compared to untreated sample, after 15 days of storage.

The defined peak of dried sample of treated and untreated guava at 0 days and peak observed between 21.5 to 23.8 degree at 2θ in all treatments which indicate the presence of sugar in sample. At 15 days of storage period, there were not more changes in all samples. XRD analysis of treated and untreated apple at 0 days and 15 days. In initial days high peak ranges between 20 to 30 degree in A0 and A3 which indicates the semicrystalline structure of control and apple treated with CuO NPs.

TESTING OF HYPOTHESIS

$H_0$1: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on TSS value of all treatment.

From table 4.5(a) and 4.5(b), it was observed that synthesized nanoparticles have significant effect on the TSS value of sample ($p<0.05$) during the storage period which indicates null hypothesis was rejected and simultaneously proven

$H_0$2: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on pH Value of all treatment.

It was observed from table 4.6(a) and 4.6(b) that pH value of sample treated with nanoparticles were not much effected during the storage period of 15 days ($p<0.05$) which implies that null hypothesis was rejected and proven simultaneously.

$H_0$3: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Acidity Value of all treatment.

From table 4.8(a) and 4.8(b) it was observed that the nanoparticles have significant effect on the acidity value of treated sample during the storage period of 15 days, hence null hypothesis was rejected and simultaneously proven.

$H_0$4: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Specific Gravity of all treatment.

From table 4.9(a) and 4.9(b) it was observed that the nanoparticles have significant effect on the specific gravity of treated sample during the storage period of 15 days, hence null hypothesis was rejected and proven simultaneously.

$H_0$5: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Reducing sugar of all treatment.

It was observed from table 4.10(a) and 4.10(b) that reducing sugar of sample treated with nanoparticles were not much effected during the storage period of 15 days ($p<0.05$) which implies that null hypothesis was rejected and proven simultaneously.

$H_0$6: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on water content of fruit sample.

The water content of the treated sample was not much effected during the storage period as compared to treated sample ($p<0.05$) as observed in 4.7 (a)and 4.7 (b)which proven that null hypothesis was rejected.

$H_0$7: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on Microbial Count of all treatment.

It was observed from table 4.11(a) and 4.11(b) that the microbial count of treated sample was less as compared to untreated sample which indicates that the nanoparticles have significant effect during the storage period ($p<0.05$) which implies that null hypothesis was rejected and proven simultaneously.

$H_0$8: There is no effect of synthesized nanoparticles (ZnO, MgO and CuO) on overall acceptability of all treatments.

From table 4.14 it was observed that the overall acceptability of treated sample was not much effected as compared to treated sample during the storage period which indicates that null hypothesis was rejected and proven simultaneously

CONCLUSION

In light of the aforementioned findings, it can be concluded that the shelf life of foods improved by using synthesized ZnO, CuO and MgO nanoparticles which was made by using sol gel process (A chemical method) and Nano packaging. Apple and guava coated with ZnO + Wax, CuO + Wax and MgO +Wax and Apple and Guava Juice treated with ZnO, CuO, MgO proven best in maintaining the storage life of sample which was tested by using various parameter. Nutritional Component, physico-chemical characters i.e. specific gravity, TSS, sugar, acidity, ascorbic acid, water loss etc. and Phytochemical parameters were almost maintained till the end of the experiment. The Microbial growth shown in sample at the end of the experiment and the morphological structure was not much change during the storage period of sample. According to this study, ZnO nanoparticles were more successful as preservatives than CuO and MgO nanoparticles.