

Eco-friendly food preservation: Studies on the synergistic preservative effect of Chitosan and soap nut solution.

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Abstract

Chitosan (CS), the antimicrobial, biopolymer with film forming nature, has been used as a topical preservative for food items like vegetables, fruits, and even for sea food.¹In order to have different choices for preservation; we need to search for more materials with such properties. Soap nut (SN), whose botanical name is *Sapindus mukorossi*, seems to have similar properties. It is saponin, the major component present in soap nut, which is responsible for its antimicrobial and film forming property. There are many reports to support that up to a certain limit saponin can be used for edible purpose.²In this study the synergistic preservative effect of chitosan and soap nut aqueous solutions on fruits and vegetables was evaluated. At first, the antimicrobial effect of CS, SN and [CS+SN] in the film form was studied against *Staphylococcus aureus* and *Escherichia Coli*. As expected the film CS+SN exhibited more antimicrobial activity with 25 mm and 23mm zone of inhibition against *Staphylococcus aureus* and *Escherichia Coli* respectively. The potential of CS+SN as a preservative was evaluated by spraying a known concentration on different regional fruits like lemon, banana, tomato and orange. Different concentrations of [CS+SN] - 250ppm and 125ppm, along with CS-500ppm, SN-500ppm and water, were compared, where water served as the control. Fruits sprayed with [CS+SN]-250ppm solution, showed significant delay in the change of weight loss, decay percentage, pH, as compared to control fruits. It also maintained better visual quality than CS and SN coated as well as control samples. These findings suggest that [CS+SN], with its synergistic preservative potential will maintain the fruit quality and lead to better acceptance by consumers. Thus

Chitosan along with soap nut solution will be a cost effective, eco-friendly and easy to utilize preservative system.

Key Words:- Eco-friendly, edible preservation, chitosan, soapnut, synergistic preservative effect, topical coating

Introduction

Natural way of preserving food is the need of present situation as number of untreatable diseases is growing day by day. The ill effects of utilizing chemicals, synthetic polymers and radiations to preserve food are threatening our livelihood. We need alternative natural ways of preserving food for a sustainable living. Traditionally salt, turmeric, tamarind, oil, and lemon were added to food in order to preserve them in the form of pickles.

For preservation of fruits and vegetables which are easily perishable commodities topical application of preservative will be preferred. A preservative should be selected in such a way that it should preserve the colour, texture, flavour and nutrients present in the fruit or vegetable. At the same time it should be harmless even if it is present in it. Biopolymers are being explored to find out their potential as natural preservative. As they can form film, that will act as a shield and protect the food from microbes.

Chitosan (CS), the ³⁻⁸edible polysaccharide, derived from marine waste is being studied for this purpose. It has ⁹antioxidant, antibacterial and antifungal properties. It can be dissolved in vinegar, which is nothing but naturally derived acetic acid. Dilute solutions of chitosan with concentrations ranging from 500-1000 ppm to 1-2% are utilized. These solutions can be sprayed on the fruit or it can be dipped into the solution, to give a coating of chitosan.

The demerits of chitosan is that if high concentrations were used the film restricts the respiration of the fruit. In order to enhance its property and also to utilise a new material for preservation, soapnut (SN), with botanical name *Sapindus mukorossi* is used. Many research works have been published on the ¹⁰⁻¹⁴antimicrobial and anticancer activity of *Sapindus mukorossi*. But not even a single report has been published about the preservative effect of soapnut. Traditionally this natural surfactant has been used for bathing and washing purposes as it exhibits amphiphilic nature. ¹⁵The hydrogel nature of chitosan and soapnut will be an added feature to enhance their activity as topical preservatives.

In the present study for the first time soapnut (SN) solution was studied for its preservative effect along with chitosan (CS) solution. They were used as topical preservative agents. The hydrogel ¹⁶⁻¹⁷nature of CS, SN, [CS+SN] was evaluated based on the values obtained for the % equilibrium water content of the materials in the film form. The antimicrobial effects of CS, SN, [CS+SN] films were also evaluated against *Staphylococcus aureus* and *Escherichia Coli*. The potential of [CS+SN] as topical preservative in different concentrations viz. 250 ppm and 500 ppm was compared with CS (500 ppm), SN (500 ppm) and water. For this purpose different regional fruits like lemon, tomato, orange and banana were sprayed with the above mentioned solutions. Properties like change of weight loss, decay

percentage fruit gloss and pH were evaluated at regular time intervals.

Materials and Method

Sapindus Mukorossi (Soap nut), lemon, orange, banana, tomato were purchased from grocery stores in Chennai, India. Chitosan (MMW) was purchased from Aldrich (CAS 44-8869) with a deacetylation percentage of 75–85%, with Brookfield viscosity 20 cps, and used as received. Acetic acid (glacial, 99-100%), Sodium bicarbonate were purchased from Merck (India) and used without further purification. Double-distilled water was used for the preparation of all solutions throughout the study.

Preparation of Chitosan solution(CS):

A homogeneous stock solution of 2% w/v of chitosan in 0.5M aqueous acetic acid was prepared by stirring the solution at 65°C for 16 hrs. From the stock, dilute solutions of 125, 250 and 500 ppm concentrations were prepared using double distilled water.

Preparation of Soapnut solution (SN):

The pericarp of Sapindus Mukorossi was dried under sunlight and it was ground into a fine powder of 40 mesh size using the laboratory mill. 20% w/v of aqueous solution of the Sapindus Mukorossi was prepared by stirring overnight. The extract was filtered through a plastic tea strainer to remove all unextractable matter. The percentage of water soluble matter in the extract was estimated by gravimetric method. The pH of the SME solution remained the same before and after extraction (pH= 7.2) and it was found to contain 8 % solid. A stock solution of 1% w/v was prepared and from this further dilutions of 125, 250 and 500 ppm concentrations were prepared.

Preparation of [CS+SN] solution:

The [CS+SN] solution were prepared by simply mixing chitosan and soapnut solutions. Table 1 gives the data on the preparation of different concentrations of [CS+SN] solutions using CS and SN solutions.

Chitosan + Soapnut	Chitosan (CS)	Soapnut (SN)
[CS+SN]125	125 ppm	125 ppm
[CS+SN]250	250 ppm	250 ppm

Table 1 Preparation of [CS+SN]

Preparation of CS, SN and [CS+SN] films:

15ml of 1% w/v of CS, SN and [CS+SN] solutions were stirred separately in three different beakers along with 0.1 ml of 25% glutaraldehyde, adjusted to pH 7 with sodium bicarbonate solutions were cast into films and dried using vacuum desiccator. The photographs of CS, SN and [CS+SN] films are shown in Fig. 1.

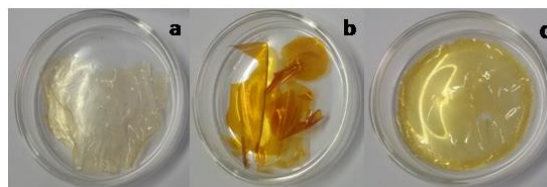


Fig. 1 The photographs of a) chitosan (CS) b) Soapnut (SN) c) [CS+SN] films

Equilibrium water content of CS, SN and [CS+SN] films:

The equilibrium water content (EWC) of CS, SN and [CS+SN] films were measured by the weight difference between the swollen hydrogel film and the dehydrated film as described previously.

This is expressed as

Antibacterial activity

Well diffusion assay (Eloff, 1998)

Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 h growing bacterial cultures (*Staphylococcus aureus* and *Escherichia coli*) were swabbed on it. Then, the test samples was been placed on the nutrient agar plate using sterile forceps. Chloramphenicol was used as standard. The plates were then incubated at 37°C for 24h. After incubation the inhibition diameter was measured.

Topical application of CS, SN and [CS+SN] solutions on the fruits

Regional fruits like lemon, orange, banana and tomato were washed with tap water and the excess water on the surface was absorbed by tissue paper. The fruits were placed into a 500 ml plastic container. The CS-500ppm, SN-500 ppm, [CS+SN]-125 & 250 ppm and water were sprayed onto the fruits until wet. Such spraying was done in every alternate day. The observation of morphological features, fruits decay over time was recorded in a notebook and also photographed every day by a digital camera.

Fruit quality studies

a) Decay percentage

The decay percentage of coated and uncoated fruit was calculated as the number of decayed fruit divided by initial number of all fruit multiplied by 100.¹⁸

b) Weight loss

The fruit samples (3 fruit) were weighed at day 0 and at the end of each storage interval. The difference between initial and final weight of fruit was considered as total weight loss at each of the storage interval

$$\% \text{ EWC} = \frac{\text{weight of water in hydrated gel}}{\text{total weight of hydrated gel}} \times 100$$

and calculated as percentage on a fresh weight basis.¹⁹

c) Determination of fruit gloss

Fruit visual appearance was evaluated subjectively by 5 persons. Fruits gloss was evaluated on a 0 to 10 scale in which

0 = no gloss and 10 = very glossy.

d) pH

The change in the pH of the fruits used for evaluation was measured from the juices obtained from the fruits at regular intervals.

Results and Discussion

Chitosan exhibits hydrogel properties due to the presence of groups like $-\text{OH}$ and $-\text{NH}_2$ along the polymer backbone. This makes it hydrophilic and retains the water it. Soap nut which is an amphiphilic in nature that is it can absorb both oil as well as water. So in order to evaluate its water absorbing capacity % equilibrium water content was calculated as given in Fig. 2.

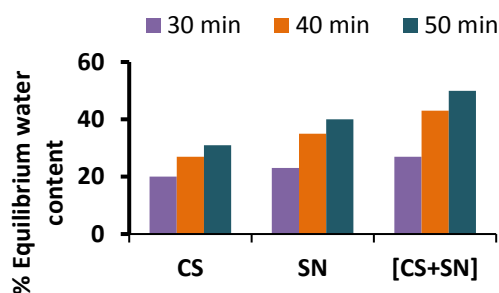


Fig. 2 The % Equilibrium water content of CS, SN and [CS+SN] films

From the Fig. 2 it is clear that compared to chitosan and soapnut, it is the combination

of both exhibits higher water intake. This quality is necessary for a preservative to absorb and retain moisture, in order to prevent the surface of the fruit from drying. When the fruit surface is fresh without drying its colour, nutrient, appearance will be enhanced.

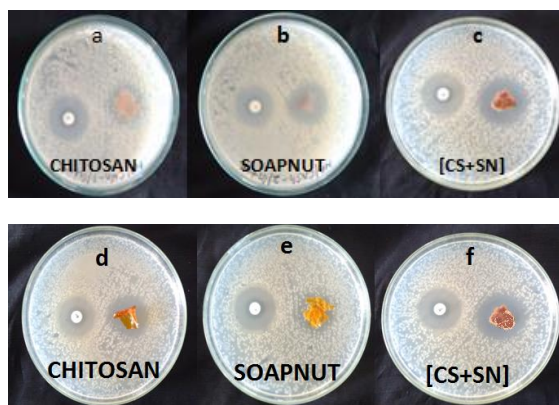


Fig. 3 The photographs showing zone of inhibition of CS, SN and [CN+SN] against (a-c) *Staphylococcus aureus* (d-f) *Escherichia coli*.

Test organism	Sample Name	Zone of inhibition (mm)
<i>S.aureus</i>	Chloramphenicol CS	26 22
	Chloramphenicol SN	23 23
	Chloramphenicol [CS+SN]	25 28
<i>E.coli</i>	Chloramphenicol CS	24 22
	Chloramphenicol SN	24 25
	Chloramphenicol [CS+SN]	24 27

Table 2 The zone of inhibition exhibited by CS, SN and [CN+SN] against *Staphylococcus aureus* and *Escherichia coli*.

The antimicrobial efficacy of CS, SN and [CN+SN] against *Staphylococcus aureus* and *Escherichia coli* is represented in Fig.3

and the Table 2 gives the values of zone of inhibition (ZOI) exhibited by the samples. From the ZOI values it can be inferred that the combined antimicrobial effect of [CN+SN] is very high when compared with the individual values. Hence the hypothesis that the combination of Chitosan and soapnut will prove to be a bio composite with good antimicrobial activity has been proved.

The decay percentage of fruits preserved is given in the Fig.4. For banana it was calculated on 15th day and for other fruits it was calculated on 25th day of preservation, as they started to decay afterwards. From the results it is obvious that the decay percentage was minimum both for [CS+SN]125 and [CS+SN]250, whereas it is maximum for water, the control.

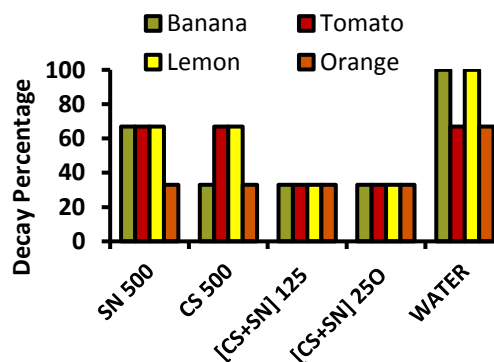


Fig.4 The decay percentage of preserved fruits

The % weight loss of preserved fruits is given in Fig. 5(a-d). From the bar diagram it is clear that banana couldn't withstand more than 15days, but other fruits stayed up to 25 days with minimum weight loss. Overall, the weight loss for [CS+SN] treated fruits is minimal for both the concentration. This could be due to the combined effect of hydrogel property of chitosan and soapnut. For soapnut alone the weight loss is more and can be compared to that of water. This

may be due to its poor film forming property despite being hydrogel.

The pH of fruits measured at various intervals is given in Table3. The pH of the fruits increased indicating the ripening of fruits. ²⁰So it is obvious that the preservative coating sprayed on top of the fruit does not interfere with the fruit ripening process.

Days	Banana	Tomato	Lemon	Orange
0	5.0	4.0	2.1	4.2
5	5.8	4.1	2.4	4.2
10	7.0	4.3	2.5	4.4
15	7.8	4.5	2.9	4.5
20	--	4.7	3.0	5.0
25	--	5.0	3.2	5.2

Table 3 The pH of fruits measured at various time intervals

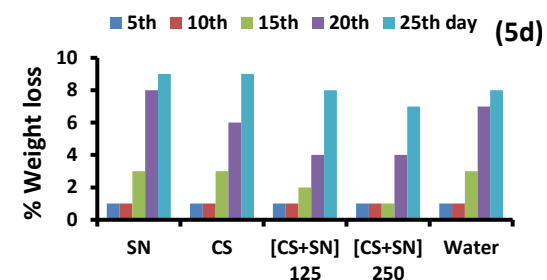
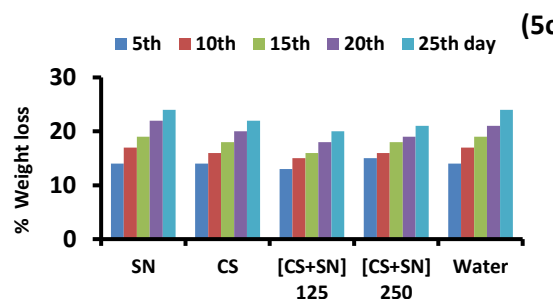
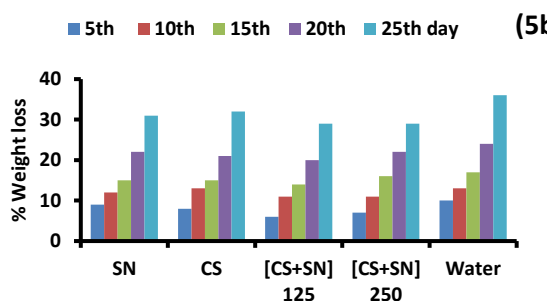
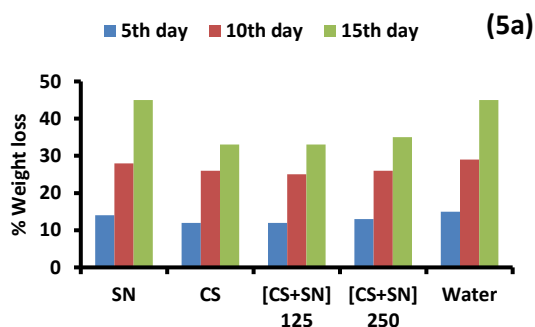
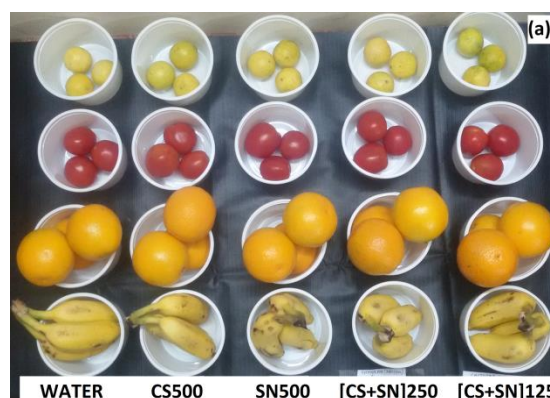


Fig. 5 % weight loss of a) Banana

b) Tomato c) Lemon d) Orange

Fruit gloss evaluation done manually also proved that [CS+SN] enhances the preservative effect. Thus among the two concentrations, it is [CS+SN] 250 more effective in preserving the fruit quality.



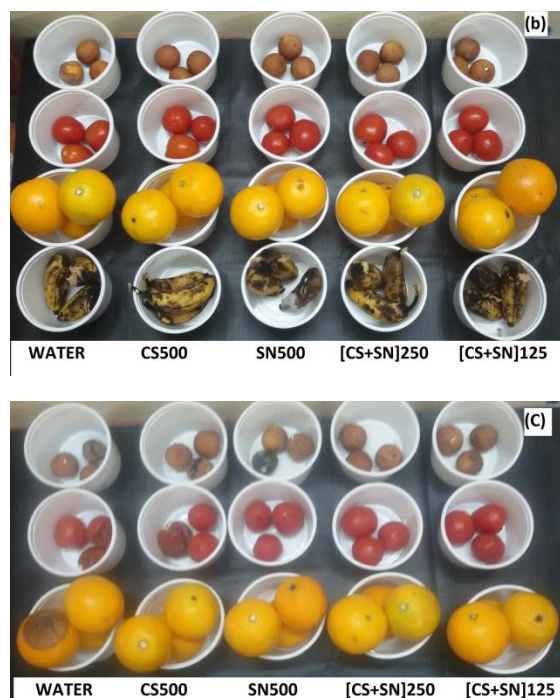


Fig. 6 The photographs of fruits a) 0 day b) 15th day c) 25th day of preservation

When the quality of the fruits were compared, orange due to its inherent juicy nature is very effectively preserved followed by tomato, then comes lemon finally the least preserved is banana, which could be visualised from the photograph given in Fig.6. Similar results have been published earlier²¹ which it has been suggest that lower doses of chitosan can be utilized as a natural preservative of fruits alternative to hazardous formalin

Conclusion

The main objective that the combination of chitosan and soapnut solution will serve as eco-friendly, edible preservative has been proved for the first time. This was proved by taking regional fruits as models. Topical application of chitosan and soapnut solution with 250 ppm concentration has shown good results. The film forming, hydrogel and antimicrobial nature of chitosan as well

soapnut is responsible for this synergistic preservative effect. Out the fruits taken it is orange which has given good results followed by tomato; lemon and least preserved is banana.

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