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Application of Active Biodegradable Film from Sago Starch and Garlic Extract in Paneer Packaging to Control Physico-chemical Changes During Storage

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ABSTRACT

In this study biodegradable packaging film was developed with sago starch and garlic extract was incorporated in it to impart an active functional role in controlling the physico-chemical changes associated with paneer storage under refrigeration. The paneer was stored in three different variants of biodegradable film and a control as Low-Density Polyethylene (LDPE) to study the effect of active biodegradable film developed in protecting the product during storage at refrigeration temperature. There were three different variants of developed film (T1, T2, and T3) where T1 was a sago starch-based film, T2 was a sago starch-based film with an edible gum coating and T3 was a sago starch-based film incorporated with garlic extract and edible gum coating. Throughout the storage trial, the average moisture content showed a significant decline ($P \leq 0.05$), although the results of control and T3 were comparable even after 12 day of storage. Although the pH values showed a decreasing trend during storage but the values in most of the trials were comparable when observed on a single day. The TBA, FFA and titratable acidity increased significantly ($P \leq 0.05$) in all the treatments and control groups with the storage but when the values were compared between control and T3 the results revealed a potential role of T3 in controlling physico-chemical changes during storage. Hence the results indicated that a biodegradable starch-based film with garlic extract and edible gum coating had promising results and could be a eco-friendly and sustainable option for the food industry.

HIGHLIGHTS

- Synthetic polymer materials of food packaging are causing huge environmental problems.
- The concept of biodegradability is environmentally conscious.
- Sago (starch) could be promising material for biodegradable film.

Keywords: Biodegradable film, Garlic extract, Packaging, Paneer, Sago starch

With a production of approximately 221 million tonnes in 2021-22, India is the world's largest producer of milk (http://dahd.nic.in/circulars/basic-animal). A significant part of this milk is used to produce paneer an important dairy product, but its shelf-life is compromised due to higher moisture content. It is required to have no more than 70% moisture content and a minimum milk fat content of 50% in dry matter (FSSAI, 2011). Because of its high moisture content (approximately 55%), paneer has a shelf-life of 1-2 days at room temperature and 5-6 days at refrigeration temperature (Kapoor *et al.,* 2021).

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Packaging is an essential component of the food industry, serving an important role in the production, preservation, distribution, and marketing of food products. Synthetic polymer materials have been widely used in virtually every field of human activity including food packaging. The world is producing twice as much plastic waste as two decades ago, with the bulk of it ending up in landfill, incinerated or leaking into the environment, and only 9% successfully recycled (https://www.oecd.org/environment/plastic-pollution.). The concept of biodegradability is not only environmentally conscious but also user-friendly, as it relies on raw materials sourced from agricultural byproducts. By adopting this approach, we can promote the conservation of natural resources while prioritizing safety and environmental friendliness. A thin layer of biodegradable materials formed on food as a coating or placed (pre-formed) on or between food components is defined as biodegradable packaging or films (Kocira *et al.,* 2021). Numerous materials are available for use as biodegradable packaging, including PHAs, PLA, starch blends, cellulose-based plastics, lignin-based polymer composites, chitin, and different nano-composites. Among them, starch is a particularly promising option due to its biodegradability, abundance, chemical inertness, and film-forming qualities. Sago, a type of starch produced from the pith of tropical palm stems, particularly those of *Metroxylon sagu,* is widely used in commercial applications and is often available in the form of small pearls.

Garlic is known to contain natural anti-oxidants that can remove reactive oxygen species (ROS) and reduce lipid peroxides and low-density lipoprotein (LDL) oxidation (Jang *et al.,* 2017). The main antioxidant compounds in garlic volatiles were found to be allicin, diallyl disulphide, and diallyl trisulphide (Batiha *et al.,* 2020). Orsuwan and Sothornvit (2018) confirmed the effectiveness of the addition of garlic oil into banana flour films as an active food packaging application to maintain the quality of dry food products. Several attempts have been made to enhance the shelf-life of paneer, which include the addition of preservatives, packaging in modified conditions like vacuum packaging, modified atmosphere packaging, dehydration and heat sterilization (Goyal and Goyal, 2016). In a study conducted on moisture content of paneer samples, it was found that during the subsequent storage period, it reduced significantly in almost all treatment samples indicating that the barrier of polyethylene bags might not be significant enough to check the moisture losses during storage (Khatkar *et al.,* 2017).

Hence the present study was designed to evaluate the potential role of biodegradable film from sago starch incorporated with garlic extract as an active ingredient to impart an effect on the physic-chemical changes associated with the storage of paneer. As biodegradable film was formed from starch hence to enhance its barrier properties edible gum was used to coat the outer side of the developed film. The role of film was studied by subjecting to *in- situ* experiment where paneer was packaged with this developed film and stored under refrigeration with concomitant assessment of physico-chemical changes during refrigeration storage.

MATERIALS AND METHODS

The investigation was conducted in the Department of Livestock Product Technology, College of Veterinary Science and Animal Husbandry, Rewa. Buffalo milk with a fat percentage of about 6% and SNF (solids not fat) of 9% was procured from the college dairy unit for the preparation of paneer. Citric acid, acetic acid, glycerol of food grade, edible gum and sago pearls used for the preparation of paneer and biodegradable film were procured from the local market. Sago pearls were ground into fine powder and used for the preparation of the desired film. LDPE films for packaging of paneer and garlic bulbs used in the experiment were procured from the local market; garlic cloves were peeled and cleaned and kept for further use. All the media, chemicals and reagents of analytical grade, glass and plastic wares required for various analyses were procured from standard firms viz., Himedia Chemicals Pvt Ltd, S.D Fine Chemicals Pvt Ltd, Central Drug House (CDH) Pvt. Ltd., Borosil Glassware etc.

Packaging variants

C: Low-density polyethylene (LDPE):

T1: Developed film from sago starch

T2: Developed film from sago starch + coating of edible gums

T3: Developed film from sago starch with incorporation of garlic extract + coating of edible gums

Preparation of packaging film T1

Preparation of biodegradable packaging film from sago starch was done by taking 10% (w/w) of finely ground sago pearl powder in a beaker with 80% (w/v) of distilled water and left aside for a few hours. This mixture was finely ground to make a uniform solution and heated at 150ºC until the mixture gets a thick texture, Next glycerol was added @5% as a plasticizer and 10% acetic acid added @5% both w/v. The solution was then poured on spreading sheets and placed for drying in a hot air oven for 20-24 hrs. at 45ºC, after proper drying, the film was peeled from spreading sheets and stored for packaging of paneer.

Preparation of packaging film T2

Edible gum from the babool plant (*Vachellia nilotica*) dissolved in equal proportion of distilled water was spread over the second batch of dried and peeled sago sheet obtained as in the T1 variant on a single side and placed in a hot air oven for 1-2 hrs. at 45ºC, thereafter they were stored for packaging of paneer.

Preparation of packaging film T3

About 100 gms of garlic bulbs were peeled, cleaned, and washed with sterile distilled water and were surface sterilized using 75% (v/v) ethanol for 60 seconds and homogenized aseptically. The homogenized mixture was filtered using a sterile muslin cloth and the extract obtained was considered a 100% concentration of the extract which was stored at refrigeration temperature till further use (Indu *et al.*, 2006).

Preparation of biodegradable packaging film from sago starch was done by taking 10% (w/w) of finely ground sago pearls powder in a beaker with 70% (w/v) of distilled water, 10% of garlic extract added and left aside for a few hours, thereafter the mixture was finely ground with the help of high power mixer grinder to make a uniform solution of the mixture. Rest of the preparation of the film was similar to the T2 variant.

Preparation of paneer and packaging in films

Paneer was prepared by the method suggested by Lamdande *et al.* (2012). It was then packaged in LDPE as Control and T1, T2, and T3 films as experiment groups and placed for storage study in refrigeration at 4 ± 1ºC.

RESULTS AND DISCUSSION

Moisture content

The moisture content (percentage) of paneer during storage in control, T1, T2 and T3 on 0 day was 50.27, 50.44, 50.49, and 50.25 respectively, which gradually showed a decrease in all the groups throughout the study period (Table 1). The study shows that there was a significant ($P \leq 0.05$) change of moisture content on each subsequent day of storage and on the last day (12th) the values observed in Control, T1, T2 and T3 were 40.63, 38.57, 41.01 and 41.04 respectively (Table 1). The study was found to be correlated with Pal (1998) and Rai *et al*. (2008) where they found a reduction in the moisture content of the paneer packed in developed films with the passing experimental days.

In all the samples under study, the moisture content was gradually reduced, however, the observation was significantly ($P \leq 0.05$) different in trials on the 6th day onwards. The study shows that utmost moisture loss was observed in the T1 group with a change from day 0 moisture content (percentage) from 50.44 to 38.57 on day 12 (Table 1) whereas a comparatively lesser change in moisture content was observed in the T2 group with 50.49 on day 0 to 41.01 on day 12 (Table 1). The results on the 9th and 12th day indicate a significant difference between paneer packaged in T1 samples and that packaged in the other two variants of developed films i.e. T2 and T3. This could be due to the coating of edible gum which acted as a good barrier for moisture loss in T2 and T3 samples. Pal *et al*. (1993) observed that the moisture content of paneer coated with wax had better moisture content than the normal paneer with no wax coating, and it correlates with the results obtained in the present study.

pH value

The pH value of paneer during storage in Control, T1, T2 and T3 on 0 day was 5.52, 5.53, 5.54, and 5.52 respectively (Table 1), which gradually showed a decrease in all groups throughout the study, although there was a slight increase in pH on 3rd day of the study which further decreased on subsequent day of study, the findings correlated with the data obtained by Arora and Gupta (1980). The study shows that there was a significant ($P \leq 0.05$) change of pH when the observations from 0 day were compared to the 12th

day in the majority of recordings, on the last day (12th) the values observed in control, T1, T2 and T3 were 5.35, 5.28, 5.44 and 5.47 respectively (Table 1).

The pH was gradually reduced in all the samples under study, however, the observation was significantly (P ≤0.05) different in trials on the 12th day only. The study shows that the maximum pH change was observed in the T1 group with a change from day 0 value of 5.53 to 5.28 on day 12 (Table 1) whereas a comparatively lesser change in pH was observed in the T2 group with 5.52 on day 0 to 5.47 on day 12 (Table 1). According to studies conducted by Rai *et al.* (2008) and Makhal *et al.* (2014) a significant decrease in the pH values of various types of paneer throughout the storage was observed.

TBA (Thiobarbituric acid) value

The TBA (mg malonaldehyde/kg) value of paneer during storage in Control, T1, T2 and T3 on 0 day was 0.233, 0.232, 0.231, and 0.235 respectively, which gradually showed an increase in all groups throughout the study period (Table 1). The study shows that there was a significant (P≤0.05) change of TBA (mg malonaldehyde/kg) value on each subsequent day of storage, The samples were evaluated, and on the last day (12th) the values observed in the Control, T1, T2 and T3 were 0.605, 0.639, 0.585 and 0.544 respectively (Table 1). The same results were obtained when studies were conducted on different food products by Witte *et al.* (1970), Okpala *et al.* (2010), Shan *et al.* (2011), Wagh *et al.* (2014) and Chauhan *et al.* (2015). Similar results were quoted by Osruwan *et al.* (2018) where garlic oil was incorporated in packaging film which played an important role in antioxidant activity against the DPPH radical.

With time the TBA value was gradually increased in all the samples under study, however, the observation was significantly (P ≤0.05) different in trials from the 3rd day onwards. The study shows that the maximum change in TBA value was observed in the T1 group with a change from day 0 TBA value from 2.32 to 0.639 mg malonaldehyde/kg on day 12 (Table 1) whereas a comparatively lesser change in TBA value was observed in T3 group with 0.235 on day 0 to 0.544 mg malonaldehyde/kg on day 12 (Table 1). In a similar study it was observed that the TBA value of the control (LDPE) sample when compared to T1 (edible film with clove oil) and T2 (edible film with oregano oil), throughout the storage period, the TBA value for the control sample changed significantly (P≤0.05), in comparison to treatments during storage (Karunamay *et al.,* 2020). On comparing T2 and T3 samples there were constantly lower values noticed in T3 samples which became significant (P ≤0.05) on the last day of storage. Even the T2 samples stored in packaging material coated with edible gum had lower values of TBA in comparison to T1 samples which could be attributed to oxygen and light transmission differences with coating of gum on T2 samples in comparison to T1 where gum coating on the film was not done. The TBA level has been identified as an indicator to detect deteriorative changes in food owing to lipid oxidation, according to Witte *et al*. (1970). This indicates the lipid oxidation of the product which during storage deteriorates and makes the food unfit for consumption. Here due to the anti-oxidant properties of garlic extract the TBA value was better in the T3 group when compared with the T1 and T2 group having no garlic extract.

FFA (Free fatty acid) value

The FFA (%) value of paneer packaged during storage in control, T1, T2 and T3 on 0 day was 0.227, 0.228, 0.226, and 0.227 respectively, which gradually showed an increase in all groups throughout the study (Table 1). The study shows that there was a significant (P≤0.05) change of FFA (%) value on the subsequent day of storage, the samples were evaluated, and on the last day (12th) the values observed in control, T1, T2 and T3 were 0.377, 0.393, 0.365 and 0.337 respectively (Table 1). The study was found to be correlated with Sindhu *et al.* (2000) and Rai *et al.* (2008) where they found an increase in the FFA content of the paneer with the storage period.

FFA content gradually increased in all the samples under study, however, the observation was significantly (P≤0.05) different in trials on the 9th day onwards. The study shows that the utmost FFA content was observed in the T1 group with a change from day 0 FFA content of 0.228 to 0.393 on day 12 (Table 1) whereas a comparatively lesser change in FFA content was observed in the T3 group with 0.227 on day 0 to 0.337 on day 12 (Table 1). This may have been due to lower lipolysis in T3 samples owing to antioxidant and antimicrobial effect of garlic extract. The present study correlated with the results of Rai *et al.* (2008) and Boghra *et al.* (1997).

Table 1: Effect of active biodegradable film from sago starch and garlic extract on physico-chemical properties of the packaged paneer during refrigeration storage

Treatment	Storage days				
	0	3	6	9	12
			Moisture content (%)		
C	$50.27^{A} \pm 0.82$	$47.08^{B} \pm 1.59$	$44.05^{abC} \pm 1.51$	$41.70^{aD} \pm 1.38$	$40.63^{aD} \pm 1.17$
T1	$50.44^{A} \pm 1.11$	$45.65^{B} \pm 1.80$	$42.88^{bC} \pm 1.94$	$39.22^{cD} \pm 1.65$	$38.57^{cD} \pm 1.28$
T2	$50.49^{A} \pm 0.77$	$45.89^{B} \pm 2.61$	$44.38^{abC} \pm 1.84$	$42.30^{aD} \pm 1.58$	$41.01^{aD} \pm 1.28$
T3	$50.25^{A} \pm 0.83$	$47.70^{B} \pm 1.66$	$45.13^{aC} \pm 1.48$	$42.83^{aD} \pm 1.37$	$41.04^{aE} \pm 1.32$
			pH values		
C	$5.52^{B} \pm 0.07$	$5.62^{A} \pm 0.08$	$5.60^{A} \pm 0.07$	$5.49^{B} \pm 0.01$	$5.35^{bC} \pm 0.03$
T1	$5.53^{BC} \pm 0.06$	$5.66^{A} \pm 0.07$	$5.60^{AB} \pm 0.07$	$5.50^{C} \pm 0.07$	$5.28^{cD} \pm 0.06$
T2	$5.54^{BC} \pm 0.07$	$5.62^{A} \pm 0.02$	$5.57^{B} \pm 0.02$	$5.49^{DC} \pm 0.07$	$5.44^{aD} \pm 0.03$
T3	$5.52^{B} \pm 0.07$	$5.67^{A} \pm 0.04$	$5.64^{A} \pm 0.09$	$5.53^{B} \pm 0.08$	$5.47^{aB} \pm 0.02$
			TBA (mg malonaldehyde/kg) values of the packaged paneer		
C	$0.233^{E} \pm 0.022$	$0.300^{abD} \pm 0.011$	$0.416^{aC} \pm 0.048$	$0.526^{bB} \pm 0.023$	$0.605^{aA} \pm 0.013$
T1	$0.232^{E} \pm 0.020$	$0.312^{aD} \pm 0.012$	$0.445^{aC} \pm 0.027$	$0.560^{aB} \pm 0.025$	$0.639^{aA} \pm 0.030$
T2	$0.231^{E} \pm 0.019$	$0.294^{bcD} \pm 0.011$	$0.356^{bC} \pm 0.028$	$0.492^{cB} \pm 0.021$	$0.585^{bA} \pm 0.012$
T3	$0.235^{E} \pm 0.022$	$0.281^{cD} \pm 0.012$	$0.335^{bC} \pm 0.019$	$0.463^{cB} \pm 0.031$	$0.544^{cA} \pm 0.022$
			FFA (%) values		
C	$0.227^{D} \pm 0.003$	$0.250^{C} \pm 0.009$	$0.266^{C} \pm 0.002$	$0.339^{abB} \pm 0.031$	$0.377^{abA} \pm 0.019$
T1	$0.228^{D} \pm 0.003$	$0.252^{C} \pm 0.007$	$0.26^{C} \pm 0.005$	$0.351^{aB} \pm 0.015$	$0.393^{aA} \pm 0.017$
T2	$0.226^{E} \pm 0.004$	$0.248^{D} \pm 0.016$	$0.261^{C} \pm 0.007$	$0.329^{abB} \pm 0.013$	$0.365^{bA} \pm 0.010$
T3	$0.227^{D} \pm 0.003$	$0.242^{CD} \pm 0.025$	$0.260^{C} \pm 0.022$	$0.308^{bB} \pm 0.010$	$0.337^{cA} \pm 0.011$
			Titratable acidity (%)		
C	$0.36^{E} \pm 0.04$	$0.45^{aD} \pm 0.03$	$0.52^{aC} \pm 0.06$	$0.60^{bB} \pm 0.04$	$0.72^{bA} \pm 0.04$
T1	$0.37^{E} \pm 0.04$	$0.46^{aD} \pm 0.02$	$0.54^{aC} \pm 0.04$	$0.64^{aB} \pm 0.01$	$0.74^{aA} \pm 0.02$
T2	$0.37^{E} \pm 0.03$	$0.43^{aD} \pm 0.03$	$0.51^{aC} \pm 0.02$	$0.58^{aB} \pm 0.02$	$0.69^{abA} \pm 0.01$
T3	$0.35^{E} \pm 0.04$	$0.40^{bD} \pm 0.02$	$0.48^{bC} \pm 0.05$	$0.53^{cB} \pm 0.02$	$0.66^{cA} \pm 0.03$

Means (Mean ± SD, n=6) with different superscripts in lower case in a column and upper case in a row differ significantly ($P \leq 0.05$) for a single parameter. C = Control, T1 = Film from sago starch, T2 = Film from sago starch + coating of edible gum, T3 = Film from sago starch with garlic extract incorporated + coating of edible gum.

TA (Titratable acidity) value

The titratable acidity (%) of paneer packaged during storage in control, T1, T2 and T3 on 0 day was 0.36, 0.37, 0.37, and 0.35 respectively, which gradually showed increased in all groups throughout the study (Table 1). The study showed that there was a significant ($P \leq 0.05$) change in titratable acidity on each subsequent day of storage, the samples were evaluated, and on the last day (12^{th}) the values observed in control, T1, T2 and T3 were 0.72, 0.74, 0.69 and 0.66 respectively (Table 1). The increased titratable acidity could be due to an increase in the microbial count with the storage period resulting more acid production. The findings here are in line with the pH values i.e. the samples having lower pH values have comparatively higher titratable acidity values and co-related with the findings of Verma and Khan (2009) for paneer and Yadav *et al*. (2019) for paneer with thyme herbs added. In a study it was found that TA of paneer samples increased with storage and after 45 days, the TA in the samples packed in different atmospheric packaging systems rose to 0.69, 0.62, and 0.66, respectively (Rai *et al.,* 2008).

With time the titratable acidity was gradually increased in all the samples under study, however, the observation was significantly (P ≤0.05) different in trials from the 3rd day onwards. The study showed that the highest titratable acidity (%) was observed in the T1 group with a change from day 0 values from 0.37 to 0.74 on day 12 (Table 1) whereas a comparatively lesser change in titratable acidity (%) was observed in T3 group with 0.35 on day 0 to 0.66 on day 12 (Table 1). The results compared from 3rd day onwards constantly indicate a significantly lower TA for T3 samples in comparison to other variants. This could be attributed to the anti-microbial effect of garlic extract T3 group which was found to have appreciably lower titratable acidity compared to the T1 group even after 12 days of storage. The results obtained were correlated with the study conducted by other workers (Chawla, 1981; Shukla *et al*., 1984; Sachdeva and Singh, 1990).

CONCLUSION

A starch-based biodegradable film prepared with garlic extract and edible gum could be used for packaging fresh paneer. The garlic extract improved the antioxidant property and retarded the deterirative changes in FFA and TA; thereby improving the keeping quality of the product. The coating of edible gum on the biodegradable film improved barrier properties; improved moisture retention in the product. This starch-based biodegradable film incorporated with garlic extract and coated with edible gum can be an excellent alternative to synthetic petroleum-based LDPE film; as it revealed appreciable features in controlling the physico-chemical changes during refrigeration storage. Hence it is concluded here that the developed film had promising results and has the potential to maintain the quality of paneer stored under refrigeration.

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