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Research Article

Fabrication and Characterization of Non-Conventional Starch Based Biofilms Employing Different Plasticizers

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Abstract

Increasing stress on environmental awareness has promoted the use of alternative strategies for common petrochemical based plastic sources to environmentally friendly biofilms and packaging materials. Food by products and underutilised indigenous plants sources are rich in biopolymers which can naturally decompose. The present study focusses on preparations of bioplastics from unconventional starch sources of litchi seed and churkha tuber as novel initiative to combat environmental pollution along with valorisation of undervalued plant sources and agrobased wastes. Ternary blend films of poly vinyl alcohol, starch, plasticizers like (glycerol, sorbitol, mannitol, propylene glycol, polyethylene glycol), citric acid prepared by casting technique produced best result. Comparative study of test samples (TH11, TH12, TH13, TH14 etc) with standardized thin films (TH1, TH2, TH3 etc.) showed variation in appearance, water activity, thickness, since the starch differ in their compositions, gelatinization and gelation time and temperature. High amylose containing litchi seed starch forms firm and more flexible films in comparison to low amylose containing churkha tuber starch. Maximum hygroscopicity was recorded in case of TH1 (77.4%) while thickness was maximum in case of TH12 (0.18 mm). Micro structural analysis clearly showed the nature of crosslinking between starch molecule and different plasticizers. The sensory analysis of food packed with the best among the formularized thin films showed mild change in taste while the other parameters remain same as normal. Better acceptability was in case of TH12 wrapped food rather than TH1. Thus, fabrication of biodegradable packaging is considered as the most sustainable alternative for food preservation.

Keywords: Biomaterials; Biopolymers; Bioplastics; Agro waste management; Environmental sustainability; Ternary blend films

Introduction

In this present century food packaging industry is dominated by petroleum-based packaging materials which includes polyethylene, polystyrene, polyamide, polyethylene, polyethylene terephthalate, etc. due to its excellent structural property, performance, cost, barrier property, aesthetic quality. However, this fossil fuel-based packaging materials is considered as the major cause for anthropogenic environmental pollution in every phase of life cycle from monomer synthesis to disposal in landfills. The contribution of synthetic plastic materials alone is about 400 million ton in total waste generation every year (Maraveas *et al.*, 2020). Recent global trend is to maintain environmental sustainability by shifting from petrochemistry to bioeconomy for preventing environmental pollution caused by toxic chemical released by fossil fuel-based packages (Silva *et al*.,2019). Thus, the production and development of novel biobased polymeric films is considered as the most innovative, promising and emerging matrices in food packaging. Every year food processing industry releases a huge number of wastes like fruit seed, fruits shells, peels, husks, protein isolates, oil seed cakes etc. which if not properly disposed can cause environmental pollution (Maraveas *et al.*, 2020). These agro based wastes are huge sources of biomaterials like protein, polysaccharides, minerals etc. that can be utilized in product formation and development (Thakur *et al.*, 2021). Thus, new environmental approach has been undertaken for development of green materials like biopolymers, biodegradable polymers, biobased plastics from agrobased waste materials in order to substitute non-ecofriendly synthetic packaging materials (Ramesh *et al.*, 2021). Among the biopolymers starch is the most abundantly found polysaccharide from plant source which is widely utilized in preparation of ecofriendly bio material-based wrappers and packages (Zhang *et al.*, 2018).

Till date starch from various unconventional and conventional sources are utilized in preparation of biobased packaging films, but it was the first-time litchi seed starch and native starch of churkha tuber was utilized in biofilm preparation (Food packaging, 2022). Starch is the most abundant polysaccharide available in seed, roots, fibre as food reserve (A Text Book of Organic Chemistry,2011). Starch or amylum is a polymeric carbohydrate comprising glucose units joined by glycosydic linkage. Starch comprises of two glucose chain: amylose (linear), amylopectin (branched) with different structural and functional property (Biochemistry, 2013). The gelatinization and gelation property of starch is utilized in preparation of bio based thin films by casting technique along with plasticizers (Talja *et al*., 2007). Litchi (*Litchi chinensis)* seed kernels are most abundant source of highly soluble amylose rich starch which is unconventional in origin. The starch granule of litchi seed is elliptical in shape with gelatinization starting at 40°C (Litchi, 2022). While churkha *(Dioscorea pentaphylla*) is one of the unexplored wild tubers with excellent nutritional and economical importance belonging to dioscoreaceae family. The starchy edible tuber churkha is widely distributed in tropical and sub-tropical region of the world (Kumar *et al*., 2017).

The main aim of the study is to utilize socioeconomically less important unconventional starch source of litchi seed and churkha tuber in fabrication of ecofriendly bio films which could effectively substitute for unsustainable petroleum-based food packaging materials and an innovative strategy for reutilizing the agro based waste materials.

Materials and Methods

Litchi seeds (*Litchi chinensis*) obtained from Baruipur farm Kolkata, Churkha tuber (*Dioscorea pentaphylla*) obtained from Jhargram district of West Bengal (Fig. 1).

Extraction of Starch

The technique of hydro milling was employed for starch extraction. 50 g of dried litchi seed (*Litchi chinensis*) and churkha powder (*Dioscorea pentaphylla*) was drenched in 1000 ml aquadest separately with continuous agitation for 6-8 hours. The slurry was filtered through cotton mesh (150 mm) followed by washing in deionized water and left to combine at 4°C for 24 hours. The supernatant was drained out and crude starch at bottom was subjected to washing, followed by oven drying at 65°C (Palacios-Fonseca *et al*., 2013).

Estimation of Amylose Content

The amylose content was estimated by using Iodine-binding procedure as described by Juliano (1981) with certain modifications (Juliano *et al*., 1981). 250 mg sample was taken in 100 ml beaker to which 1 ml ethanol and 10 ml of 1 (N) NaOH were mixed. The solution was subjected to boiling for 10 minutes followed by cooling at room temperature. 2.5 ml of this solution was poured in a 50 ml test tube to which 20 ml distilled water was added. Then, 1-2 drops of 1% phenolphthalein indicator were added along with dropwise addition of 0.1 (N) HCl until pink colour disappeared. After which freshly prepared 1 ml iodine reagent was mixed and the reading of optical density was taken at 590 nm in spectrophotometer after volume makeup in a 50 ml volumetric flask (Thilakarathna *et al*., 2017).

Litchi seeds	Litchi seed starch	Churkha tuber	Churkha tuber starch
			

Fig.1: Litch seeds and Churkha tuber samples used in the study

Gelation Property

Powered sample was taken separately at different concentration ranging 5-25% w/v and mixed properly. The test tubes were subjected to heating in water bath at 85ºC for 30 minutes followed by rapid cooling under running cold tap water. The test tubes were further cooled at 4ºC for 2 hours. The lowest gelation concentration was determined as that concentration when the sample did not drop or slip from the inverted test tubes (Chowdhury *et al*., 2012).

Fabrication of Biofilms

Two different methods were employed for biofilms preparation by using solvent casting technique (Table 1). In first method, 1 g starch in 17 ml water was subjected to gelatinization along with 2 ml plasticizer (glycerol, mannitol, sorbitol, polyethylene glycol, propylene glycol) at 70-80℃ followed by casting on glass plate and oven dried at 40℃. The dried films were carefully peeled off and stored in desiccator (Pareta *et al*., 2006). In second one, film composition was prepared by mixing two different gelatinized solution containing 1.4 g of polyvinyl alcohol in 15 ml of water in boiling condition and another one containing 1.4 g of starch in 15 ml water along with 0.5 g citric acid and 1 g of plasticizer followed by continuous swirling over hot plate at 80-85℃. The homogenized mixture was casted on glass plate (25 cm X 25 cm) followed by drying at room temperature. The dried films were peeled off from plate and stored in desiccator for further characterizations (Wu *et al*., 2017).

Table 1: Various thin film combinations with different starch sources Characterization of biofilms.

Combination	Code	PVA(g)	Starch(g)	Citric acid(g)	Plasticizer				
					Glycerol	Sorbitol	Mannitol	PEG	PG
Food grade corn starch (CCS1)	TH1	1.4	1.4	0.5	1ml	-	-	-	-
	TH2	1.4	1.4	0.5	-	1g	-	-	-
	TH3	1.4	1.4	0.5	-	-	1g	-	-
	TH4	1.4	1.4	0.5	-	-	-	1ml	-
	TH5	1.4	1.4	0.5	-	-	-	-	1ml
Laboratory grade corn starch (CCS2)	TH6	1.4	1.4	0.5	1ml	-	-	-	-
	TH7	1.4	1.4	0.5	-	1g	-	-	-
	TH8	1.4	1.4	0.5	-	-	1g	-	-
	TH9	1.4	1.4	0.5	-	-	-	1ml	-
	TH10	1.4	1.4	0.5	-	-	-	-	1ml
Litchi seed starch (LSS)	TH11	1.4	1.4	0.5	1ml	-	-	-	-
	TH12	1.4	1.4	0.5	-	1g	-	-	-
	TH13	1.4	1.4	0.5	-	-	1g	-	-
	TH14	1.4	1.4	0.5	-	-	-	1ml	-
	TH15	1.4	1.4	0.5	-	-	-	-	1ml
Churkha tuber starch (CTS)	TH16	1.4	1.4	0.5	1ml	-	-	-	-
	TH17	1.4	1.4	0.5	-	1g	-	-	-
	TH18	1.4	1.4	0.5	-	-	1g	-	-
	TH19	1.4	1.4	0.5	-	-	-	1ml	-
	TH20	1.4	1.4	0.5	-	-	-	-	1ml

Moisture Content

The moisture content of was evaluated by using a digital weighing scale. All samples were weighted before W_i g (initial weight) and subjected to oven drying until a constant weight W_f g (final weight) was achieved (Tarique *et al*., 2021).

Percentage of Moisture = $(W_i-W_f)/W_i$ X100

Where, W_i= initial weight of the Sample; W_f=final weight of the Sample

Thickness

Screw gauge measurement technique was followed for measuring the thickness of the films. The biofilm thickness was measured with 0.001 mm sensitivity by using an advanced micrometer. Measurement was taken in five distinctive portion of each sample biofilms and the mean value was estimated for determining the thickness (Tarique *et al*., 2021).

t = M + n X L.C (mm)

Where, t= Thickness; M= Linear Scale reading (mm); n= Circular Scale Reading (mm); L.C= Least Count (mm) (Singh *et al.*, 2018).

Microscopic Studies

Microscopic observation of the biofilms was carried out under high magnification compound microscope (Leica ICC 50). Microscopic observation was done to observe the superficial morphological crosslinking of the starch films (Wu *et al*., 2017).

Packaging of Food Product

In this study biofilms were used as a packaging material. Food product (Peanut Chikki) was packed with the prepared biofilms and kept in air tight container for 21 days. Shelf-life study and sensory characteristics of the food was evaluated at an interval of 7 days (Garcia *et al.*, 2012).

Sensory Analysis of Packaged Food

The food product (Peanut chikki) was packed with biomaterial based thin films was supplied to the panel members for sensory characteristic observation. 9-point hedonic scale was used for sensory characteristic evaluation (Garcia *et al.*, 2012).

Results and Discussions

Amylose Content of Starch

The amylose content of litchi seed starch is higher **(**25 g/100 g) in comparison with churkha tuber starch **(**15 g/100 g**)**. The Table 2 shows the amylose and amylopectin content of litchi seed starch and churkha tuber starch in comparison to other unconventional starch sources (Romero-Bastida *et al*., 2005).

Table 2: Comparative study on the amylose and amylopectin of sample with other unconventional starch sources

Sample	Amylose (g per 100 g of sample)	Amylopectin (g per 100 g of sample)
Litchi seed starch	25	75
Churkha tuber starch	15	85
Mango	28.7	71.3
Banana	35	65
Okenia	26.1	73.9

The amylose content of litchi seed starch and Churkha tuber starch was determined by using standard curve.

The physicochemical property and the way in which the amylose and amylopectin chain are dispersed, is entirely dependent on the botanical origin of the starch (Bajaj *et al*., 2018). Recent studies on the effect of starch on structure, plasticization and properties of films prepared by solvent casting technique revealed that structure of amylopectin films is sensitive and dependent on preparation condition while amylose films are crystalline, mechanically stable and less effected by plasticization of the polyols (Myllarinen *et al*., 2002). Again, high amylose containing starch generally form strong and stiff films by linking linear chain with hydrogen bonding. Thus, high amylose containing litchi seed starch possess better film forming property in comparison to churkha tuber starch. This was similar to the findings of Tarique *et al* and Bertuzzi *et al*, who observed that high amylose content formed stronger and more crystalline films in arrowroot and corn starch respectively (Bertuzzi *et al*., 2007).

Gelation property of Starch

The gelation property of litchi seed starch starts from 15% concentration and at 25% concentration complete gelatinization takes place. While Churkha tuber starch possess least gelation property which show gluey tendency above 20 % concentration (Table 3).

The variation in gelation property was observed probably because of the difference in amylose and amylopectin content of starch. The structural features have complex effect on gelation property. In the present study better gelation capacity was observed in case of litchi seed starch than churkha tuber starch. The causal reason for this is the high amylose content of litchi seed starch which is associated with inhibiting swelling in initial stage, increasing pasting temperature, lowering peak viscosity and improving onset viscosity that determines the ability to form firm gel network while high amylopectin content of churkha tuber starch promotes swelling but lowers the gelation property by causing thinning of starch paste during heating. Similar feature was recorded by Jane *et al.*, who observed that waxy starch with no amylose content reflects lower ability to form gel network (Jane *et al.*, 1999). Furthermost according to the findings of Lim *et al*, absence of phospholipid and lipid in roots and tubers starch are responsible for lower resistance to shear thinning, lower set back viscosities as in comparison to cereal starch (Lim *et al*., 1994). In addition to low amylose content this property might be responsible for the low gelation property of churkha tuber starch in comparison to litchi seed starch. Again, according to previous study minimum amylose-amylopectin ratio of 0.43 is required for formation of gel network. At lower amylose content the starch gel easily disrupts by heating, this might be responsible for least gelation capacity of churkha tuber starch (Sasaki *et al*., 2005).

Study on Prepared Thin Films

Variation in the psychochemical property of the films obtained probably because of the use of different method in film preparation. In the solvent casting technique only by utilizing different plasticizers along with starch produced mostly brittle, inflexible, unplasticized films which torn while removing from the glass plate. This finding was similar to the observation of Talja *et al*, Tarique *et al*, who prepared starch films with potato starch and arrowroot starch respectively when plasticizer content was less than 20%. While Ternary blend films of PVA, starch, citric acid combinations along with plasticizers provided best result (Pareta *et al*., 2006). Among all biofilms prepared the result obtained with **TH12** was most promising in overall appearance. The influence of different film forming technique on the nature of biofilms prepared by utilizing various plasticizers is summarized in Table 4 & 5.

The structural difference of the films prepared might be due to variation in nature of crosslinking between starch molecule and plasticizers used (Hanani *et al.*, 2014). The first method resulted in formation of brittle, rigid, wavy films which torn into bits while removal from glass plate. This might be due to insufficient molecular interaction between starch and different plasticizers, resulting in inadequate separation between the macromolecular chain of starch in presence of strong intramolecular hydrogen bonds (Tarique *et al*., 2021). The phase separation between starch and plasticizer matrix also might be responsible for the stickiness of film forming solution resulting in unplasticized film formation (Talja *et al*., 2007). In the second method of film preparation low molecular sized plasticizers might have slide into the space between the molecules of the starch polymeric chain boosting the molecular mobility by decreasing the strength of hydrogen bonds resulting in flexibility of plasticized films (Tarique *et al*., 2021). The addition of PVA in the second method might have improved the nature of cross linking between starch molecules and plasticizer, while citric acid addition increased the compactness and stability of starch films (Pareta *et al*., 2006). The rate of gelatinization and the nature of starch films produced is dependent on the concentration of amylose and amylopectin. Starch with high amylose content form strong and flexible films while starch with high amylopectin forms weak and brittle ones (Tarique *et al*., 2021). Thus, the biofilms from litchi seed starch with high amylose (25 g/100 g) content were more flexible in comparison to Churkha tuber starch biofilms with low amylose content (15 g/100 g). The film forming ability was more appreciable in case of litchi seed starch than churkha tuber starch, which might be due to the variation in amylose content.

Table 3: Gelation property of litchi seed starch and churkha tuber starch.

Samples	**Concentrations**				
	5%	**10%**	**15%**	**20%**	**25%**
Litchi seed starch	- (No visible gel formation)	-	-/+ (Slight onset of gelation)	+ (Viscous gel formation)	+
Churkha tuber starch	-	-	-	-	-/+

Table 4: Characteristics of the thin films obtained by different film forming techniques.

Plasticizers	**Method 1**	**Method 2**
Glycerol	Fragile, brittle, non-peelable films	Flexible, non-sticking, peelable films
Sorbitol	Wavy, inflexible, brittle, slightly sticking, non-peelable films	Flexible, elastic, easily peelable, non-sticking films
Mannitol	No film formation	Flexible, non-brittle, easily peelable films
Propylene glycol	No film formation, phase separation	Flexible, easily peelable, elastic films
Polyethylene glycol	No film formation, phase separation	Flexible, non-sticky, plasticized films

Table 5: Different thin films from unconventional and conventional starch sources

TH3	TH12	TH16	TH11
			

Moisture Content

The study on water activity of the prepared films reveals that, maximum moisture content was recorded in case of TH1 (77.4%) while minimum moisture content was observed in case of TH12 **(**3.18%). Comparative study of thin films from litchi (*Litchi chinensis*) seeds starch and churkha (*Dioscorea* pentaphylla) tuber starch reveals that the moisture content was high in case of churkha tuber starch. In this present study moisture content of biofilms from litchi seed starch ranges between 3.18%-20.34% with different plasticizers while the moisture content of churkha tuber starch films varies from 15.23%- 30.45%. This is in contrary with the findings of Hazrati *et al* who observed that the moisture content of *D. hispida* starch-based biofilm was 10.46% with sorbitol as plasticizer (Hazrati *et al.,* 2021).

Difference in starch compositions and wide variety of plasticizer used might be responsible for variation in moisture content which determine the effectiveness of the starch films to be utilized as packaging material. The moisture content of biofilms might be dependent on the presence of hydrophilic and hydrophobic component in film forming solution, increase in the hydrophilic constituents increases the moisture content of starch films. The highly hydrophilic nature of corn starch (food grade) and glycerol might be responsible for high moisture content of the film obtained from it. The hydroxyl group present in glycerol might have form strong attraction with water which enable them to hold water by forming hydrogen bond within the structure (Tarique *et al*., 2021). The Fig. 2 and Fig. 3 represents the moisture content of various combinations with different plasticizers.

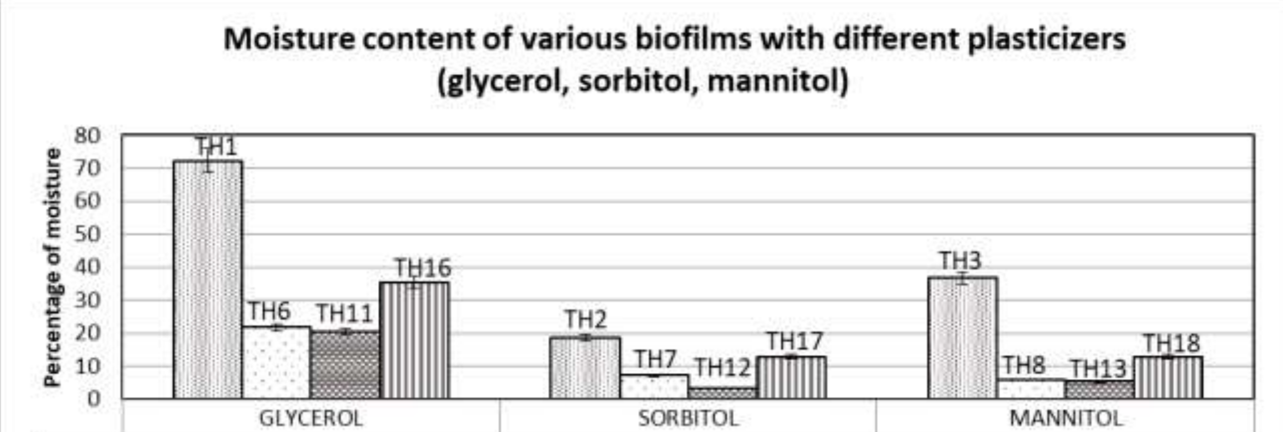
Thickness

Variation in the thickness of the films was noticed by utilizing different film forming solution and methods in preparation of biofilms. The plasticizer plays significant role in upsetting and restructuring the macromolecular polymeric arrangement of starch converting all free space into thickness. Thus, different plasticizers used is associated is with causing variation in thickness of biofilms. The film forming process is associated with formation of new strong bonds by destroying the original intermolecular bonds of starch chain which reduces the free space (Hazrati *et al.,* 2021).

The thickness generally increases with increase in solid content, thus addition of citric acid along with PVA might be responsible for increase in thickness of films in second method. Maximum thickness of 0.18 mm was in case of TH12 while minimum of 0.13 mm in case of TH5 was recorded. The Fig. 4 represents the comparative study on thickness of the best combinations. Mostly the biofilms plasticized with glycerol and sorbitol were best in appearance. Thus, the thickness study elucidated the comparison between glycerol and sorbitol plasticized biofilms with different starch sources. In case of conventional starch sources, the thickness of biofilms plasticized with glycerol was higher in comparison to sorbitol plasticized biofilms, while this was reversed in case of unconventional starch sources. The thickness might be due to physical interaction between the polymeric matrix of starch, PVA, citric acid and carboxylation of citric acid with alcoholic hydroxyl group of PVA. Increase in molecular adhesion between litchi seed starch PVA and sorbitol might have resulted in increase in thickness of the film (Pareta *et al*., 2006). The difference in starch compositions and variation in size of starch granule might be responsible for the wide variations in thickness of the films**.** High amylose content and large molecular size in litchi seed starch might be responsible for increase in thickness. This was similar to the findings of Romero-Bastida,C.A *et. al* who observed mango and banana starch produced thicker films than okenia starch by utilizing same film forming solution due to the difference in amylose content.

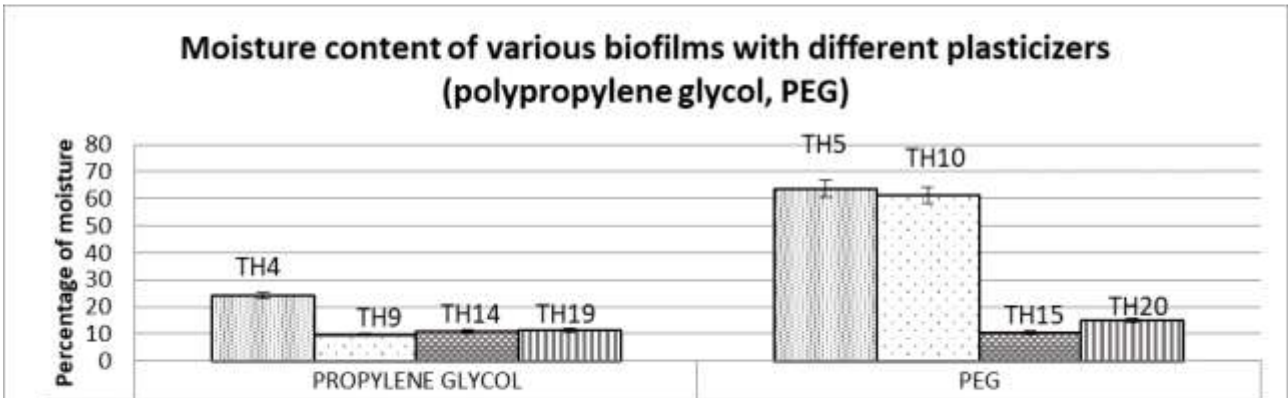
Microscopic Observation

Microstructural analysis and study on structural morphology determines the nature of crosslinking between starch granules and plasticizers used. The structural topography of ternary blended films revealed that the surface was homogeneous, smooth, continuous with no pores on surface which indicated that the plasticizers and citric acid might have improved the binding between starch and PVA. Similar finding was also reported by Wu *et al* and Parvin *et al,* who prepared starch-PVA blended films from rice starch and corn starch respectively (Parvin *et al.,* 2010). The change in starch micro domain to continuous phase from dispersed phase reveals the miscibility of starch in PVA. The microscopic view of the cross section also revealed the composite film surface was free from phase separation interface between starch and PVA, projections and wrinkles (Pareta *et al*., 2006). The variation in colour was observed depending on the starch sources and natural interaction between the iodine and starch granules (Fig.5).



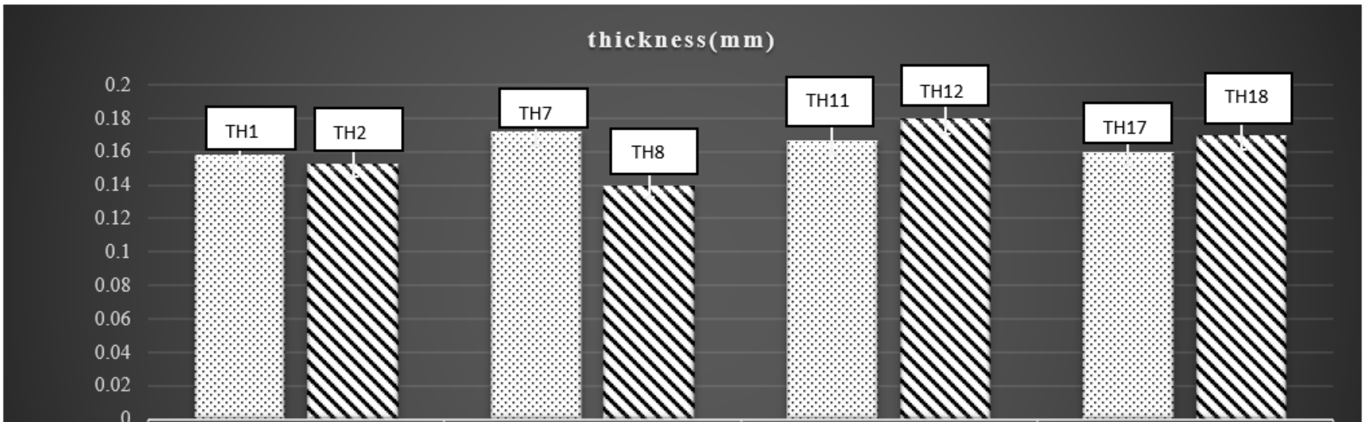
	GLYCEROL	SORBITOL	MANNITOL
CCS1	72.24	18.45	36.66
CCS2	21.85	7.14	5.86
LSS	20.34	3.18	5.33
CTS	35.41	12.9	12.9

Fig. 2: Comparative study on moisture content of starch films with glycerol, sorbitol, mannitol as plasticizer



	PROPYLENE GLYCOL	PEG
CCS1	24	63.64
CCS2	9.68	61.38
LSS	10.93	10.41
CTS	11.29	15.09

Fig. 3: Comparative study on moisture content of starch films with propylene glycol and PEG as plasticizer



	CCS1	CCS2	LSS	CTS
glycerol	0.158	0.172	0.167	0.16
sorbitol	0.153	0.14	0.18	0.17

Fig. 4: Study On Thickness of The Thin Films.

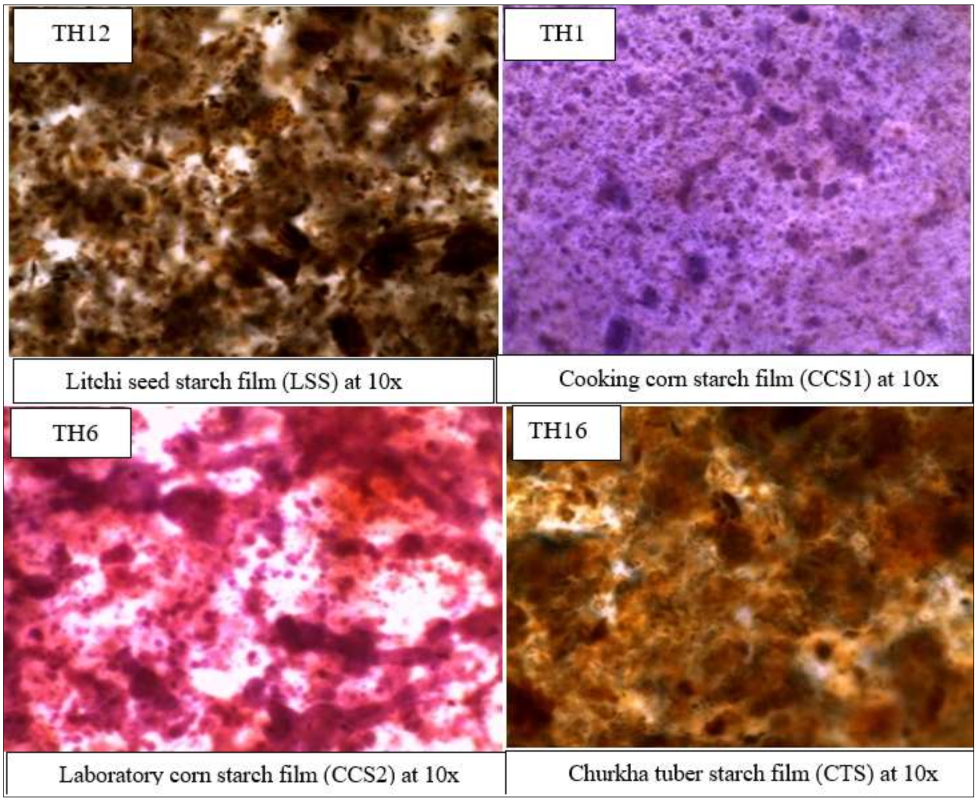


Fig. 5: Microscopic image of the cross section of the starch-based biofilm

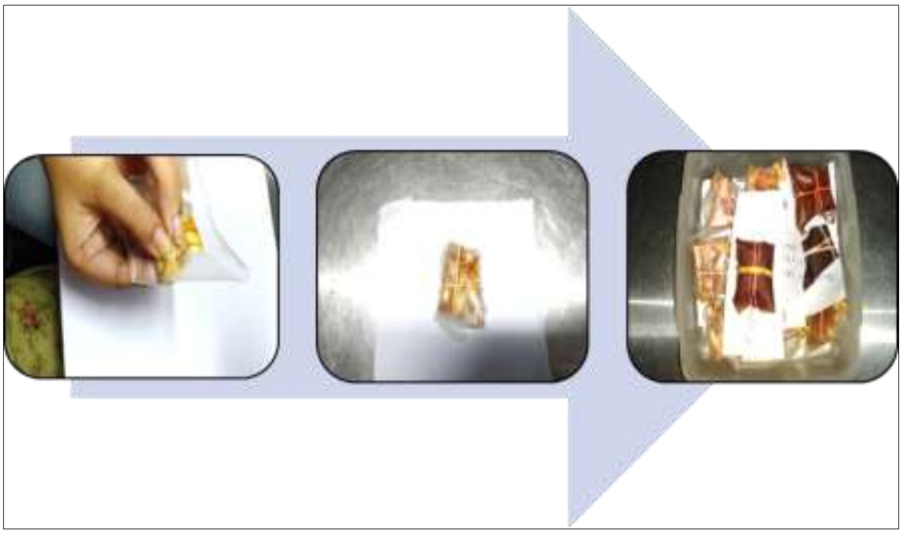


Fig. 6: Flow chart showing the process of wrapping the food in biobased films

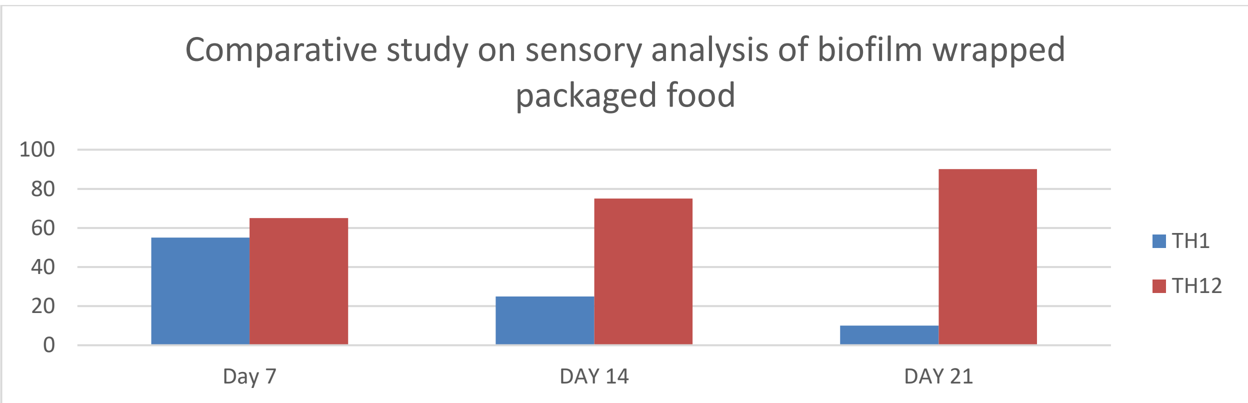


Fig. 7: Sensory analysis of biofilm wrapped packaged food.

Food Packaging Material Study

The apparently best film among the control (TH1) and test (TH12) category was selected and utilised in packaging of peanut chikki (Fig.6).

Sensory analysis of food (peanut chikki) at an interval of 7, 14, 21 days using biobased thin films as packing materials revealed slight sourness of taste which may be due to the presence of citric acid in film forming solution while the color, hardness, odour, brittleness of the film remained normal (Fig. 7). No visible growth of microorganism was noticed on the surface of the food. In terms of acceptability TH12 wrapped food created better response in comparison to TH1.

Conclusion

This study is based on the novel approach to use biomaterial based thin films from unconventional starch and their application as food packaging material. Among the methods utilized in preparation of starch films, ternary blend films by combining PVA and citric acid produced best result. High amylose containing litchi seed starch possess better film forming ability as in comparison to low amylose containing churkha tuber starch. Wide variation in moisture content and thickness was noticed due to difference in composition of film forming solution. Microscopic studies revealed nature of crosslinking of starch with plasticizer. The sensory analysis of food material packaged with biobased thin films showed significant quality maintenance in comparison with standard packaged food. From this study it can be concluded that bio polymer based thin films can effectively substitute non-biodegradable packing materials.

Authors' Contribution

Dr. A Chatterjee designed the research plan, analysed the data; critically revised and finalized the manuscript. S Laha, performed the experiments, collected the data and prepared the manuscript. Final form of manuscript was approved by both authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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